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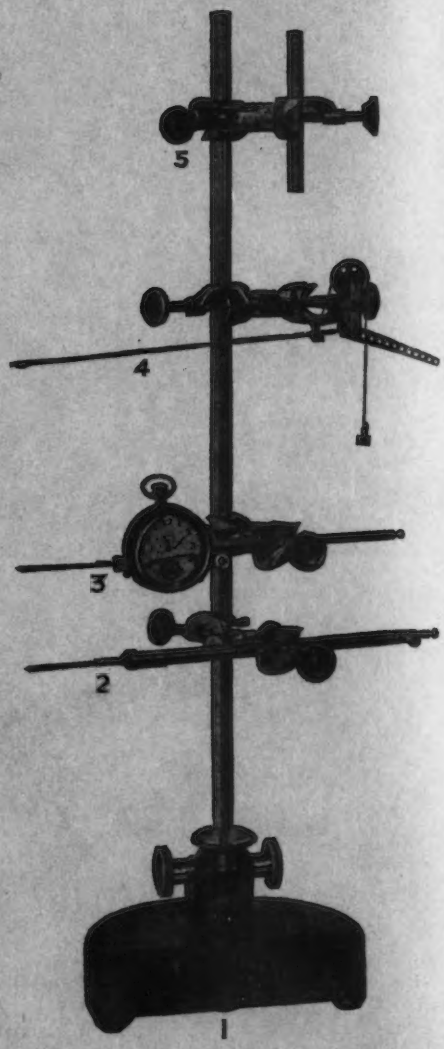

JOSEPH BECKER

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MICRO-INJECTION STUDIES OF CAPILLARY PERMEABILITY

II. The Relation Between Capillary Pressure and the Rate at Which Fluid Passes Through the Walls of Single Capillaries

EUGENE M. LANDIS

From the Department of Physiology, Medical School, University of Pennsylvania, and the Marine Biological Laboratory, Woods Hole, Mass.

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Previous work on the vessels of the frog mesentery by the micro-injection method (Landis, 1926a) has indicated a balance between the average capillary blood pressure and the osmotic pressure of the plasma proteins. The fact that the average pressure in the arteriolar capillary is above—and in the venous capillary is below—the osmotic pressure of the plasma colloids is regarded as evidence favoring the hypothesis of Starling (1896). According to this view fluid leaves the blood stream when capillary pressure rises above the osmotic pressure of the plasma proteins, to which the normal endothelium is impermeable; and enters the blood stream when the capillary pressure falls below that level.

This paper deals with further experiments designed to study the permeability of the capillary wall in a quantitative manner, with control of capillary pressure. The experiments fall into two groups. In the first the movement of fluid through the capillary wall is identified indirectly by injecting solutions of certain easily visible dyes; in the second the rate of fluid passage inward or outward through the endothelium is measured directly by the respective dilution or concentration of the corpuscles in the capillary. Both methods provide further evidence of a more direct character favoring the view that fluid movement depends upon the level of capillary pressure in relation to the effective osmotic pressure of the plasma colloids.

I. The passage of dye solutions in relation to capillary pressure and dilatation. The rate of movement of dye solutions through the

capillary wall has been believed to depend on various chemical and physical qualities of the dyes used (Wittgenstein and Krebs, 1926; Schuleman, 1917; Schimidzu, 1922; Pohle, 1924). With any single dye solution, however, the state of the capillary wall itself has usually been regarded as the chief factor in determining the rate of passage. No account of the possible effect of changes in capillary pressure has been found in the literature.

Rogowicz (1885) observed that a dye introduced into the saphenous vein passed into the tissues more rapidly whenever active hyperemia was induced by stimulation of the vaso-dilator nerves or by division of the vasoconstrictor fibers. Krogh (1922) states that brilliant vital red and Chicago blue 6B, both relatively indiffusible substances, pass very slowly through the normal capillary wall; with the production of hyperemia or stasis, however, the vessels are quickly surrounded by a deeply stained zone. Capillary pressure, though not measured, was believed by Krogh to be a factor of slight importance. It was concluded that owing to a mechanical stretching of the endothelium the dilated capillary wall becomes more permeable even to colloidal substances. The active hyperemia resulting from local heating of the skin (Okuneff, 1924) produces in the affected area a more marked staining of the tissues after intravenous injection of trypan blue. Anitschkov (1924) also attributed this effect to local dilatation of the capillaries.

The micro-injection studies before cited (Landis, 1926b) have shown, however, that with an increase in the rate of flow, such as accompanies the dilatation and active hyperemia produced by heat or very dilute urethane, there is a marked rise in capillary pressure. It seems possible therefore that the increased passage of dye under these conditions may be related to this rise of capillary pressure rather than to the "stretching of the capillary wall" in dilatation. The experiments to be described were undertaken with the view of determining whether the passage of certain dyes is related to the level of capillary pressure, as would be expected from the Starling hypothesis, or to capillary diameter as suggested by Krogh.

Material and methods used in the dye experiments. The capillaries in the mesentery of the frog (Rana pipiens and R. catesbiana) were used throughout. The animals were pithed in the brain, the spinal cord remaining intact. The mesentery was delivered through a lateral incision, which had been cauterized lightly to prevent blood loss. After exposure the preparation was kept moist by a stream of Ringer's solution of pH 8.2 to 8.4 from a reservoir mounted on the frog board. The capillaries were observed through a 16 mm. objective and a Zeiss photomicrographic ocular.

Single capillaries were perfused at known pressures by means of the micro-injection apparatus previously described in detail. Flow in the vessel to be perfused was temporarily stopped at *a* (fig. 1) by pressure with the microscopic glass rod, to prevent the blood plasma from mixing with the

injected solutions. The micro-pipette, filled with the dye solution and attached to the apparatus for measuring pressure, was inserted at *b*. A slow flow of dye through the vessel was produced (fig. 2) by raising the pressure on the solution in the micro-pipette 1 to 2 cm. above the observed capillary pressure. In this way the corpuscles and plasma were removed and the fluid in the observed capillary consisted only of a constantly renewed Ringer's solution containing a known amount of dye.

Preliminary experiments (1927a) indicated that vessels of like diameter, or those arising from the same arteriole, frequently appear to present very different grades of permeability to dye solutions. It was found that these variations depend upon the level of capillary pressure, which is different not only in adjacent capillaries but changes in the same vessel from moment to moment. In each injection it was necessary therefore to measure pressure in the observed capillary at least before and after, and frequently also during, the period of perfusion.

As soon as the dye appeared in barely visible amount (fig. 2) outside the capillary wall the perfusion was stopped and the time recorded. Capillary diameter was measured with an ocular micrometer, during or immediately after the micro-injection. The passage of dye was observed at a distance of 200 to 500 micra from the point where the micro-pipette pierced the capillary in order that the results might not be complicated by injury of the wall. Finally in many instances the circulation was permitted to return to the vessel in order to observe whether the wall had been injured by the perfusion, as indicated by the abnormal loss of plasma and the production of stasis.

It was necessary to exercise extreme precaution against injury to the blood vessels in manipulating the mesentery during preparation. It was found that poor circulation, due either to hemorrhage or to pithing of the cord, leads to erratic results, since dyes pass with extreme rapidity, even at low pressure, through the capillary walls of a dead or moribund animal.

The filtration of toluidine blue in relation to capillary pressure and capillary diameter. Toluidine blue[1] is well adapted for micro-injection of this type because of its intensity of color and low degree of toxicity. Normal blood flow continues without indication of injury through capillaries stained a deep blue by long-continued perfusion. Its passage is not entirely unaccompanied by vital staining but this is relatively slight in an experiment requiring only a minimal passage through the capillary wall. The solution introduced consisted of 0.015 molar toluidine blue dissolved in standard

[1] The dyes used were vital stains prepared by the National Aniline and Chemical Company, New York, with the exception of trypan blue, which was a Grübler preparation.

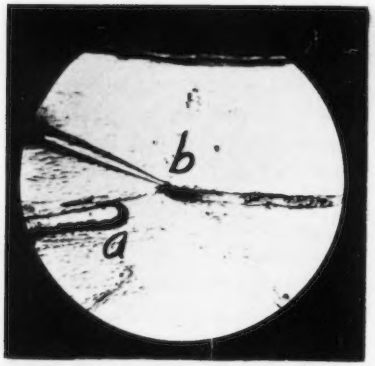

Fig. 1

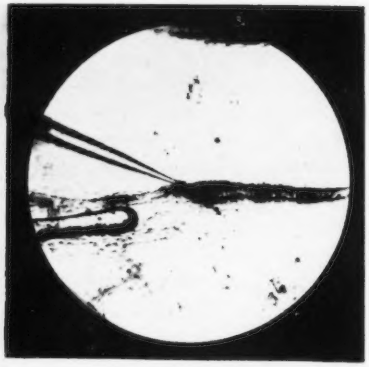

Fig. 2

Fig. 3

Fig. 4

Fig. 1. Photomicrograph (× 80) showing perfusion of a capillary with 0.005 M brilliant vital red in Ringer's fluid. *a* is the blunt glass rod by which circulation is stopped; *b* is the tip of the micro-pipette through which the dye is introduced and capillary pressure measured.

Fig. 2. The same capillary two minutes later, after the dye has passed through the wall. Capillary pressure was 18 to 19 cm. water.

Fig. 3. Photomicrograph (× 80) showing capillary after stoppage of flow at *a* with blunt rod. Within 2 minutes the corpuscles had moved inward as shown, indicating filtration of fluid. Capillary pressure was 26 cm. water.

Fig. 4. Photomicrograph (× 70) showing a second capillary similarly treated. Corpuscles had not changed their position at the end of 2 minutes. Capillary pressure was 9.5 cm. water.

frog Ringer's solution. Protocol 1 describes a typical experiment in which a series of capillaries were thus injected:

Protocol 1. June 6, 1926. Medium sized bull-frog pithed in brain only and prepared without loss of blood. Circulation in the mesentery very rapid and pressure generally high. Injected toluidine blue 0.015 molar in Ringer's solution.

Inj. 1. Mean pressure 30 cm. water, the capillary being directly connected to an arteriole
Dye appeared outside the capillary wall in one area after 5 seconds perfusion, and outside the entire vessel after 10 seconds
Diameter 19 micra
Pressure at end still 30 cm.

Inj. 2. Venous capillary pressure 16 cm.
Dye outside after 90 seconds
Diameter 32 micra
Pressure 16 cm.

Inj. 3. Pressure 12 cm.
Diameter 33 micra
Dye outside the capillary after 105 seconds
Pressure 14 cm.

Inj. 4. Pressure 18 cm.
Dye outside 22–26 seconds
Diameter 25 micra
Pressure 18 cm.

Inj. 5. Pressure 10 cm.
Dye outside in 150 seconds
Diameter 25 micra
Pressure 11 cm.

Inj. 6. Pressure 8 cm.
No visible passage after 300 seconds' perfusion
Diameter 30 micra
Pressure 7.5 cm.

Using this method 116 injections of individual capillaries were made in the mesenteries of 27 frogs. The observations have been arranged in groups according to *a*, the diameter of the capillaries injected, and *b*, the capillary pressure at which the perfusion was carried out.

In table 1 the results are arranged according to the diameter of the injected vessels. When perfusion had been continued for five minutes without passage of dye the experiment was ended and the time recorded as 5.0+ minutes. In the table there is shown the number of observations in each group, the variation in time of perfusion required, the average time, and finally the average pressure.

The times required ranged from 0.1 minute to 5.0+ minutes. As may be seen when the observations are arranged according to capillary diameter extremely high and low values are found in each of the groups. The figures have been plotted in figure 5 where it appears that the average time for the appearance of dye outside the vessel is fairly constant in capillaries of increasing diameter. There is no evidence therefore in these experiments of a measurable increase in permeability with dilatation of the capillary.

TABLE 1

DIAMETER OF CAPILLARY	NUMBER OF OBSERVATIONS	TIME REQUIRED FOR VISIBLE PASSAGE			AVERAGE CAPILLARY PRESSURE
		Lowest	Highest	Average	
micra		*minutes*	*minutes*	*minutes*	*centimeters*
11–14	11	0.1	5.0+	1.8	15
15–18	37	0.2	5.0+	2.3	14
19–22	11	0.2	5.0	2.4	12
23–25	28	0.4	5.0+	1.9	11
26–33	12	0.1	4.0	1.8	12
34–37	2	1.4	2.1	1.7	10

TABLE 2

CAPILLARY PRESSURE	NUMBER OF OBSERVATIONS	TIME REQUIRED FOR VISIBLE PASSAGE			AVERAGE DIAMETER
		Lowest	Highest	Average	
centimeters		*minutes*	*minutes*	*minutes*	*micra*
7– 8.5	14	4.1	5.0+	4.8	21
9–10.5	22	2.2	5.0+	3.1	24
11–12.5	26	1.0	4.0	2.2	23
13–14.5	21	0.5	3.5	2.1	20
15–16.5	14	0.5	3.0	1.5	19
17–18.5	4	0.3	1.5	0.8	23
19–20.5	3	0.3	0.7	0.5	17
21–22.5	4	0.1	0.5	0.3	16
23–24.5	4	0.2	0.7	0.5	18
25–26.5	2	0.1	0.5	0.3	17
27–30.5	2	0.1	0.2	0.15	18

An objection to this conclusion might be raised on the ground that the vessels perfused were merely capillaries of different size, not of varying degrees of dilatation. However, when the mesentery is first exposed to view practically all the vessels are constricted to such an extent that even single corpuscles have great difficulty in passing, but within ten minutes after exposure nearly all are dilated to widely varying degrees. During the hour or two following this first reaction to exposure the number of constricted vessels again increases considerably; and since it was during this

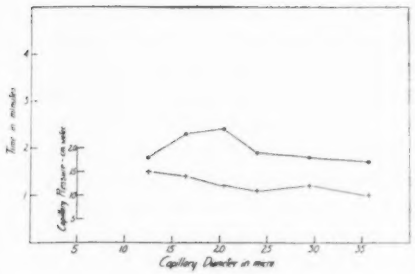

Fig. 5

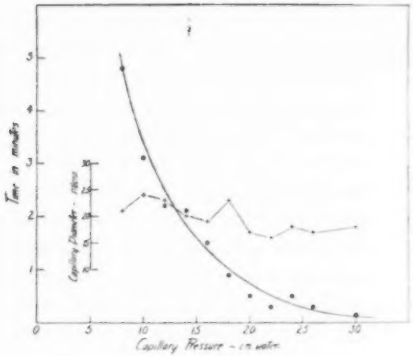

Fig. 6

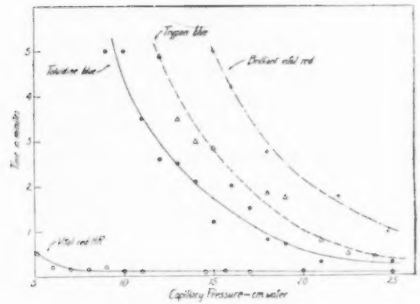

Fig. 7

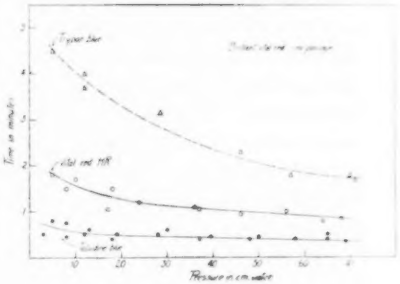

Fig. 8

Fig. 5. Chart showing the relation between capillary diameter and the rapidity with which 0.015 M toluidine blue in Ringer's fluid passes through the capillary wall.

Abscissae indicate capillary diameter in micra. Upper line designates average time of perfusion required for visible passage; lower line the average capillary pressure in each group of observations.

Fig. 6. Chart showing the relation between capillary pressure and the rapidity with which 0.015 M toluidine blue in Ringer's fluid passes through the capillary wall.

Abscissae indicate capillary pressure in centimeters water. Smooth curve shows the average time of perfusion required for visible passage; horizontal line indicates the average capillary diameter in each group of observations.

Fig. 7. Chart showing the relation between capillary pressure and the rapidity with which vital red HR, toluidine blue, trypan blue, and brilliant vital red, each in 0.005 M solution, pass through the capillary wall.

Abscissae indicate capillary pressure; ordinates show the time of perfusion required for visible passage of dye.

Fig. 8. Chart showing the permeability of collodion capillaries 8 per cent collodion in 80 per cent alcohol and 20 per cent ether) to toluidine blue, vital red HR, and to trypan blue. Brilliant vital red did not pass.

Abscissae indicate perfusion pressure; ordinates show the time required for visible passage.

period that nearly all the observations were made, the vessels injected were really in varying stages of dilatation and secondary constriction.

When, however, the results are grouped according to the capillary pressure at the time of perfusion the relation of dye passage to capillary pressure is clearly indicated, as is shown in table 2. It may be observed in figure 6 that at a pressure of 9 cm. or less in the majority of cases no dye passes through the wall even after 5 minutes' continuous perfusion. With rise in pressure there is a rapid decrease in the time required, so that at 15 cm. the average is 1.5 minutes, while at 30 cm. 10 to 15 seconds are sufficient. The average diameter of the capillaries remains fairly constant so that the correspondence with capillary pressure seems quite clear.

It appears from these results therefore that the rate of passage of toluidine blue in Ringer's solution is dependent upon the level of capillary pressure rather than upon the degree of dilatation.

Comparison of vital red HR, toluidine blue, trypan blue and brilliant vital red in their relation to capillary pressure. In order to determine whether the behavior of other substances than toluidine blue would show the same relationship to capillary pressure an identical procedure has been used with a series of vital dyes. Of those tested, some, such as rose bengale, could not be used because of the toxicity of the samples available. Others, such as methylene blue, neutral red and Congo red pass through the wall very unevenly due to their affinity for certain cellular elements outside the capillary.

The four dyes chosen for comparison were vital red HR, toluidine blue, trypan blue and brilliant vital red. Each was dissolved in Ringer's solution in the strength of 0.005 molar. The results of the capillary perfusions with these solutions are shown charted in figure 7. It seems clear that the four dyes are similarly affected by capillary pressure, in that passage is more rapid at the higher levels. They differ markedly, however, in the time required for the passage of a visible amount at any given capillary pressure.

Vital red HR (trypan red) was found by Schuleman (1917) to be one of the most diffusible members of the benzopurpurin group. When introduced into the capillary by micro-injection it passes through the wall almost immediately even at very low pressures. Its passage can be markedly delayed, however, if the colloid osmotic pressure of the dye solution is increased with gum arabic or gelatin. This effect can hardly be due to chemical combination with the colloid since diffusion into gelatin still occurs readily from these solutions, indicating the presence of dye in diffusible form. With 1 per cent gelatin solution the dye does not pass unless the pressure is in excess of 7 to 9 cm. water. As with toluidine blue there is no perceptible diminution in the permeability of the capillary wall with decrease of diameter.

Toluidine blue in the same concentration does not pass at pressures below 9 to 10 cm. Phenolsulphonephthalein and methylene blue apparently lie between vital red and toluidine blue. The former could not be used since change in reaction causes it to take on an orange tint as it passes through the wall, the latter because of its affinity for the Rouget cells.

Trypan blue in Ringer's solution requires a pressure in excess of 12 to 13 cm. for passage, filtering therefore with less ease than toluidine blue. According to other observers it passes only with moderate readiness (Edwards, 1926; Grollman, 1926) or with relative difficulty (Schulten, 1925) through the glomerular capillaries. Though passing more slowly than toluidine blue at low pressures, its rapidity of filtration is much increased by a rise of capillary pressure.

Brilliant vital red, used by Krogh and Harrop (1921) and by Krogh (1922) passes with the least facility, very little appearing outside the vessels at pressures below 14 to 15.5 cm. Even at 25 cm. a minute's perfusion is necessary to produce visible passage. Its rate of filtration, however, shows the same correlation with capillary pressure and the same lack of relation with capillary dilatation as was observed with each of the other dyes. A protocol of a typical experiment is given:

Protocol 2. December 7, 1926. Large female leopard frog, pithed in brain only. Ovaries and eggs removed after ligation of the pedicle. Circulation normal.

Inj. 1. Pressure first 16 cm., then 14 cm.
Diameter 19 micra
Passage in one portion of the vessel at 3.5 minutes, but in remainder no passage at 5 minutes

Inj. 2. Pressure 22.5 cm.
Diameter 25 micra
Passage in 2.5 minutes

Inj. 3. Pressure 15.5 cm.
Diameter 24 micra
Faint passage at 4.5 minutes, clear at 5 minutes

Inj. 4. Pressure 21 cm.
Diameter 11 micra
Passage at 2.75 minutes

Inj. 5. Pressure 14 cm.
Diameter 15 micra
None at 5 minutes

Inj. 6. Pressure 12.5 cm.
Diameter 18 to 25 micra
None at 5 minutes

Inj. 7. Pressure 12, 13, finally 13.5 cm.
Diameter 26 micra
No passage at 5 minutes

Inj. 8. Pressure 14 cm., then 16 cm.
Diameter 25 micra
Very faint passage at 4.5 minutes

Inj. 9. Pressure 18 cm.
Diameter 27 micra
Passage in 3.5 minutes

Physical properties of the dye solutions. The differences in the facility with which these four dye solutions pass through the capillary wall are apparently not related to their rate of diffusion into gelatin. Into 5, 20 and 30 per cent gelatin, toluidine blue diffuses far more rapidly than vital red HR. Trypan blue diffuses at a very slightly slower rate than vital red HR, while brilliant vital red is almost entirely indiffusible. Nor can the differences be related to the acid or basic nature of the dyes, since two acid dyes, vital red HR and phenolsulphonephthalein, pass more rapidly; while two others, trypan blue and brilliant vital red, pass more slowly, than do the basic dyes, methylene blue and toluidine blue.

According to Michaelis (1905) semi-colloidal and colloidal dyes probably exist in two states, one dissolved and the other colloidal, the proportions varying with concentration. From this property it might be expected that those dyes which apparently prevent the filtration of water below a certain pressure would show evidences of colloidal behavior. This has been found to be the case insofar as brilliant vital red, which passes only at pressures above 14 to 15 cm., produces by far the most perfect Tyndall cone when a beam of light is transmitted by the dye solution. Trypan blue, though too opaque in 0.005 molar solution to permit observation, produces a somewhat less clear cone in more dilute solution. With toluidine blue there is noted a clear beam of reddish light—the fluorescence which is described by Michaelis. In the case of vital red, however, though examined in 0.005 M concentration, no Tyndall cone was visible. This, coupled with the fact that it is the only dye which was found to pass freely at low pressures, suggests that its lack of colloidal properties may possibly explain its behaviour compared to other dyes.

Moreover, if vital red HR and trypan blue each in 0.005 M strength in Ringer's fluid are introduced together into a capillary, no dye whatever passes at pressures below 12 cm. Above this level a violet coloration results. Yet diffusion in gelatin separates out to some extent the red from the blue dye. It seems probable that water and the substances in true solution, including the vital red HR, are retained in the capillary lumen by the osmotic pressure of the colloidal portion of the trypan blue at pressures below 12 cm. water.

Comparison of capillaries and collodion tubes in relation to dye passage. To test further the osmotic properties of the dye solutions, capillaries of collodion were prepared. Threads of sugar were formed by "pulling" a plastic mass of heated dextrose. The threads were cooled and then immersed in collodion of the three grades of permeability used by Krogh (1922). After drying in the air for 3 minutes the thread of sugar, coated with collodion, was placed in 60 per cent alcohol, which dissolved out the dextrose, leaving a collodion capillary between 100 and 300 micra in diameter and about 10 cm. in length with two flaring ends measuring 3 to 4 mm. in diameter. A glass tube was tied into each end and the capillary was mounted in a holder to prevent undue tension.

The outside of the capillary was bathed in Ringer's solution while the dye solutions described above were perfused through the lumen. One area of the collodion tube was observed under the microscope as the dye solution flowed and the time for barely visible passage was recorded as for the mesenteric capillaries. Flow was maintained constant while pressure was varied between 5 and 80 cm. of water. Toluidine blue passes with greater rapidity and at lower pressures than any of the acid dyes. Vital red HR is the only acid dye of the three studied which passes through the wall of a capillary made of 8 per cent collodion dissolved in equal parts of ether and alcohol.

Capillaries made of 8 per cent collodion in 80 per cent alcohol and 20 per cent ether are far more variable in their degree of permeability and more difficult to duplicate. They permit practically immediate passage of toluidine blue, a somewhat slower passage of vital red, and a still more delayed passage of trypan blue (fig. 8). They are entirely impermeable to brilliant vital red.

It is suggestive that the acid dyes show the same order in their facility of passage as was observed with the capillary wall of the frog. The negative charge on the collodion membrane is possibly the complicating factor which delays the passage of vital red HR, trypan blue, brilliant vital red and apparently all acid dyes. Thus the wall of the collodion capillary permits the basic dye, toluidine blue, to pass far more rapidly than vital red HR. This difference between the collodion capillary and the endothelial wall of mesenteric capillaries confirms the findings of Grollman (1926) who observed that the comparison of the capillary wall with a simple collodion membrane as a filter for dyes was not justified.

It seems probable from the physical properties described above that the differences observed in the four dye solutions are to be explained on the basis of their colloidal properties and their osmotic activity with reference to the endothelium as a filtering membrane.

The passage of vital red HR injected into the blood stream. Vital red HR was the only dye to which the capillary endothelium appeared to be wholly

permeable. It can be introduced into the blood stream to a visible concentration apparently without increasing the colloid osmotic pressure of the plasma. It was therefore injected as a saturated solution into the blood passing through the ventricle until the plasma in the mesenteric vessels had a definitely pink color. One capillary was selected for observation of the passage of the dye. At the end of five minutes' observation a micro-pipette was inserted into a collateral vessel and capillary pressure measured while flow still continued.

It was found that in those capillaries where no dye passed the pressure was always below 11 cm. Where passage was rapid the pressure was almost always 14 cm. or over. In any case, diameter played a part only indirectly, since dilated capillaries with high pressure seemed quite permeable, while other dilated vessels with low pressure seemed impermeable.

In animals which had suffered severe hemorrhage and in which capillary pressure was very low there was no passage of dye. It moved only slowly or not at all through the walls of constricted vessels, where pressure is usually low. If, however, the total circulation of an arteriole was diverted, by means of the microscopic glass rod, into such a vessel thus increasing its pressure, the dye passed very quickly. Frequently during the first five minutes it did not come out equally along the entire length of any one capillary but in greater amount outside the arterial portion of the vessel.

Injury of the wall leads to a rapid filtration of the dye, often with the production of stasis, which is easily explicable on the basis of a lowered effective colloid osmotic pressure, due to the increased permeability of the injured endothelium. Application of urethane similarly causes passage at lower pressures owing to injury of the wall (Landis, 1927b). These results can best be explained on the view that there is in the plasma a certain amount of freely diffusible dye which acts as an indicator of the filtration of fluid. At capillary pressures below the colloid osmotic pressure of the plasma the flow of fluid is inward, but above this level fluid and the dissolved dye pass outward and cause staining.

This relationship holds, however, only during the first ten minutes following the introduction of the dye. After this time the results are obscured by the rapid vital staining of the connective tissue around the smaller venous capillaries and particularly the smallest venules. These are stained deeply irrespective of pressure or diameter. Nevertheless the behaviour of the dye during the first five minutes before vital staining occurs provides further qualitative evidence in favor of the importance of capillary pressure in fluid movement.

II. The direct determination of the rate of fluid movement through the capillary wall in relation to capillary pressure. In earlier experiments it was noted that after injury of the capillary a concentration of cells, indicating filtration of fluid through the wall, occurs

slowly or not at all when capillary pressure is low, and rapidly when the pressure is high. These observations have been extended to normal capillaries in such a way as to permit the measurement of the absolute rate at which fluid moves through a unit area of capillary wall at various pressures.

If the flow of a rapidly moving stream of blood is stopped by gently compressing the capillary with the blunt glass rod, in some instances the corpuscles rapidly move inward toward the closed end (fig. 3), in others they move outward, and in still others (fig. 4) no change can be observed. The forces involved are *a*, the hydrostatic pressure exerted by the blood stream at the open end of the capillary, tending to cause filtration; and *b*, the osmotic pressure of the plasma proteins in the imprisoned blood which tends to prevent filtration or to bring about absorption of fluid. According to the Starling hypothesis filtration should occur in those capillaries where hydrostatic pressure is high, absorption where it is low, and neither when the two pressures are approximately balanced. This appears to be the case.

With the capillary closed at one extremity, as long as the diameter remains constant and the corpuscles move freely with the plasma, filtration of fluid causes the corpuscles at the open extremity to move inward toward the closed end. With absorption of fluid the corpuscles at the open end of the capillary are displaced outward to make room for the added volume of fluid within the vessel.

To measure the rate of movement of the corpuscles at the free end of such a closed capillary an ocular micrometer scale, 660 micra in length and divided into 20 units, is placed parallel to the capillary. The lumen of the latter is closed by gentle pressure with the rod opposite one end of the scale (*a*, fig. 3) and the eye at the same time is fixed upon one edge of a corpuscle at the other end of the scale (*b*, fig. 3). The movement of this corpuscle is followed for a period of 60 to 100 seconds and the times at which it passes each mark on the scale are recorded by signal magnet and kymograph. At the end of the period of observation a micro-pipette is introduced into the capillary and the pressure is measured with the blunt rod still in position. Record is made of capillary pressure, the diameter of the vessel, the position of the corpuscle at the outset, and the times required for it to move past the markings of the ocular micrometer (see protocol 3 below).

Figure 9 shows the relation between the rapidity and direction of the movement of the corpuscle at the open extremity in relation to capillary pressure, which is given at the right of each curve. The experiments involve capillaries of approximately the same length and diameter in order to roughly eliminate differences in filtering area and volume change. Filtration is indicated by the inward movement of the corpuscles, which, as the chart shows, occurs at an increasingly rapid rate as capillary pres-

sure rises above 11.5 to 12.5 cm. Absorption of fluid, indicated by the outward movement of the corpuscles, becomes more rapid as pressure falls below 9 to 9.5 cm.

In each experiment the movement of the observed corpuscle is at first rapid, then becomes slower, and finally at a certain middle range of pressures ceases altogether. This apparently indicates a gradual concentration or dilution of the plasma colloids until their new osmotic pressure corresponds to the capillary pressure. Even this roughly qualitative treatment of the data makes it appear that the osmotic pressure of the plasma proteins must lie somewhere between 9 and 12.5 cm. water, and that as capillary pressure rises above this point there is filtration of fluid, and as it falls below it, absorption. This relationship can be indicated more exactly by computing the unit rate of fluid movement at various capillary pressures.

Computation of the rate at which fluid passes through the capillary wall. With the first change in the volume of the blood imprisoned in the capillary the osmotic pressure of the plasma colloids is no longer at the level normal for freely flowing blood. It is therefore the volume change at the first instant which must be used to compute the rate of filtration from unchanged blood. This is obtained by drawing for each experiment a curve of the type shown in figure 9. A tangent to this curve at zero time will give the rate at which the corpuscle was moving while the osmotic pressure of the plasma proteins was still essentially normal. This figure (micra per second) multiplied by the cross-sectional area of the capillary will indicate the rate (in cubic micra per second) at which fluid was being filtered or absorbed through the observed length of capillary by normal blood.

The area of filtering surface at zero time is computed by finding the area of the cylinder of endothelium extending from the closed end of the capillary (*a*, fig. 3) to the position of the observed corpuscle at the beginning of the experiment (*b*, fig. 3). The volume change per second divided by the filtering area (in square micra) will give the unit rate of fluid movement in cubic micra per square micron of capillary wall per second. In protocol 3 is shown in tabular form the observed values and computations of a typical experiment.

Seventy capillaries in the mesenteries of 11 frogs have provided the results shown in figure 10, in which the unit rate of fluid movement is plotted against capillary pressure, filtration being designated + and absorption −. The disproportionately large number of points on the zero line is due to the fact that in those experiments in which the corpuscles moved less than one micrometer unit (33 micra) it was necessary to record the rate of movement as zero.

The sources of error lie chiefly in the lack of absolute uniformity of

capillary diameter through the entire length of the vessel observed, and in the possibility that during the 30 to 60 seconds elapsing between the measurement of fluid movement and the determination of pressure there may be some change in the latter, though this difficulty was guarded against by observing the rate of flow in collateral vessels, which has been shown to follow faithfully the fluctuations of pressure over short periods of time.

Protocol 3. February 15, 1927. Small-sized female leopard frog. Ovaries and eggs removed after ligation of pedicle. Mesentery prepared without blood loss. Circulation moderately rapid.

CAPILLARY NUMBER	CAPILLARY PRESSURE	DIAMETER	POSITION OF CORPUSCLE	TIME	RATE OF MOVEMENT	RATE OF VOLUME CHANGE	FILTERING AREA	UNIT RATE OF FLUID
	cm. water	*μ*	*μ*	*seconds*	*μ/second*	*cubic μ/second*	*square μ*	*cubic μ/square μ/second*
1	26	18	660	0	9.4	2,390	37,300	0.064
			627	3.5				
			594	7.0				
			561	10.5				
			528	14.0				
			495	18.0				
			462	22.0				
2	20.5	27	594	0	4.7	2,690	50,400	0.053
			561	8.5				
			528	30.0				
3	15.7	22	660	0	2.2	840	45,600	0.018
			627	12.5				
			594	31.0				
			561	43.0				
4	13.3	17	495	0	0.5	110	26,440	0.004
			479	52.0				
			Remained stationary					
5	17.0	25	660	0	3.8	1,880	51,840	0.036
			627	8.0				
			594	19.0				
			561	27.0				
			528	39.0				
6	8.0	15	495	0	−1.4	−243	23,300	−0.011
			511	13.0				
			528	32.0				
7	13.7	20	495	No movement		0	31,100	0

In addition the spread of observed values is in part probably due to the fact that the blood which is imprisoned within the capillary varies according to its source and previous rate of flow. The greater volume changes are observed when the rate of flow, preceding the experiment, is rapid, or when the blood enters the capillary directly from an arteriole; since in either case there is less opportunity for previous fluid interchange. Slow flow, particularly through a long capillary, permits the blood to come partially into equilibrium with the local capillary pressure and therefore diminishes the rate of filtration observed when flow is stopped in the experiment. For this reason it seemed necessary to make all determinations in vessels in which flow was at least moderately rapid.

As indicated in figure 10 at pressures between 29 and 14.5 cm. the movement of fluid is outward in all cases. Between 14 and 12.5 cm. fluid passage occurs very slowly in an outward direction or is absent, the latter state indicating a condition of equilibrium. From 12 to 7 cm. movement is absent or inward, and below 7 cm. inward movement is observed in all cases.

At 25 cm. pressure the observed rates of filtration range from 0.045 to 0.068 cubic micra per square micron of capillary wall per second, while at 15 cm. they vary from 0.012 to 0.018. The large majority of observations between pressures of 9 and 13 cm. show no movement of fluid in either direction. At 5 cm. pressure fluid is absorbed at rates between 0.020 and 0.050 cubic micra per square micron per second.

As would be expected from Poiseuille's law, the observed points are dis-

Fig. 9. Chart showing the manner in which capillary pressure determines the rate and direction of movement of the corpuscles at the open extremity of the occluded capillary. Vessels of similar length and diameter have been chosen to make volume changes approximately the same.

Ordinates indicate movement of corpuscles in arbitrary units, each equivalent to 33 micra. With filtration of fluid the cells move inward toward the closed end; with absorption the increase in plasma volume causes the cells to move outward toward the open extremity. Abscissae show time in seconds.

Fig. 10. Chart showing the rate of fluid movement in relation to capillary pressure.

Ordinates indicate rate of fluid movement in cubic micra per square micron of capillary surface per second. Positive values denote filtration of fluid, negative values absorption. Abscissae show capillary pressure in cm. water. The equation for the line is $x = 11.5 + 178\,y$.

Fig. 11. Chart showing the marked increase in rate of passage of brilliant vital red through the walls of capillaries injured by ether to the point of stasis.

Abscissae denote capillary pressure in cm. water. Ordinates indicate the time of perfusion required for visible passage of dye.

Fig. 12. Chart comparing the rates of filtration of fluid through normal endothelium (below) and the walls of capillaries killed by 10 per cent alcohol (dots) and mercuric chloride 1:10,000 (crosses).

Ordinates indicate rate of fluid movement in cubic micra per square micron capillary wall per second. Abscissae indicate capillary pressure in centimeters of water

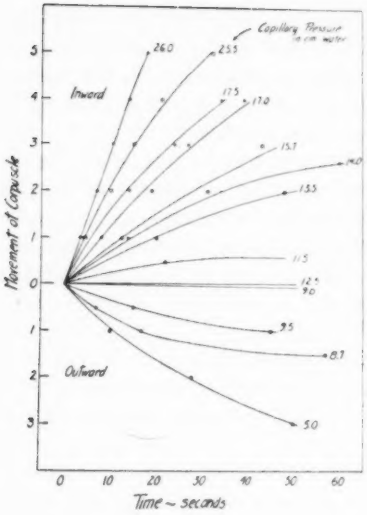

Fig. 9

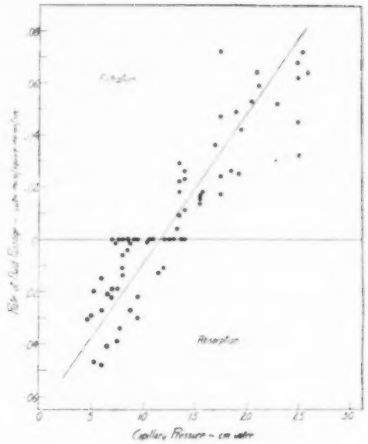

Fig. 10

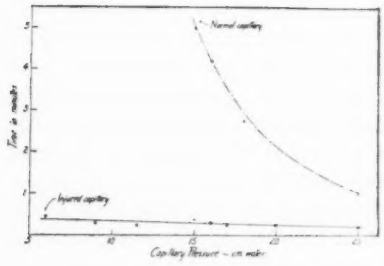

Fig. 11

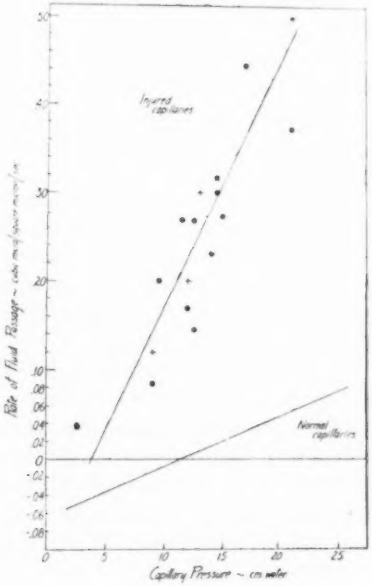

Fig. 12

tributed about a straight line. This line, computed by the method of least squares, has an equation $x = 11.5 + 178\ y$, where x is capillary pressure and y is the rate of filtration and therefore intersects the line of zero filtration at 11.5 cm. pressure. This represents the capillary pressure in cubic centimeters of water at which fluid moves neither inward nor outward. Since by definition this is osmotic pressure, it is believed that it represents the osmotic pressure of the plasma proteins against their normal filtering membrane. The slope of the line indicates the rate of movement of fluid in relation to the difference between the colloid osmotic pressure and capillary pressure, amounting to approximately 0.03 cubic micra per square micron capillary surface per second for a difference of 5 cm. water pressure.

This method can therefore be used under various experimental conditions to indicate the permeability of the capillary wall with reference to a, the rate of movement of fluid, and b, the effective osmotic pressure of the plasma proteins.

III. The permeability of the injured capillary wall determined by experiments of both types. There is evidence that following injury of the capillary wall by certain means permeability is increased to such an extent that the plasma colloids can pass with ease (Jacobj, 1921; Osato, 1922; Tainter and Hanzlik, 1924). The higher concentrations of urethane, alcohol, ether, etc. (Landis, 1927b) appear to injure the endothelium to such an extent that the plasma with its proteins is almost entirely drained from the blood in the capillary vessels, leaving solid masses of closely packed cells—the condition to which Krogh (1922) has applied the term of "stasis."

With dyes the effect of various toxic substances on the capillary wall is easily demonstrated to be in the direction of greater permeability. The toluidine blue solution above described passes through the injured wall in 5 to 6 seconds at all pressures, even those below 9 cm., at which level the normal capillary, due possibly to the semi-colloidal nature of the dye, permits no visible passage. The same can be observed with trypan blue and brilliant vital red. For the latter, figure 11 shows the time of perfusion required at various pressures to produce visible passage of dye through the walls of normal capillaries and of those injured to the point of stasis by Ringer's solution saturated with ether. The same results are obtained when the dye solution is saturated with ether and injected into normal capillaries. With injury the endothelium seems to become quite permeable to semi-colloidal dyes, as well as to the plasma colloids, at all pressures.

The method of direct measurement of fluid passage has also been applied to injured capillaries. The mesentery was exposed to a constant flow of 10 per cent alcohol in Ringer's fluid. After this had continued for 10

minutes the flow of blood through the capillary network usually became more sluggish, and the corpuscles appeared to be more concentrated, indicating an increase of permeability. When flow was then stopped, in the manner described above, in a series of single capillaries, a very rapid concentration of cells was noted, even at pressures as low as 4 to 5 cm. of water. The unit rate of filtration as determined for a moderate range of capillary pressure is indicated in figure 12. As with the normal vessels the points are distributed about a straight line, but the line is shifted to the left and the slope increased. The equation is $x = 4.0 + 26\ y$, indicating that filtration occurs at approximately seven times the rate determined for normal capillaries, for a given difference between the effective osmotic pressure of the plasma proteins and capillary pressure.

In the normal capillary the concentration of cells never proceeds to the point where practically all of the plasma is filtered off, due to the mounting colloid osmotic pressure, which finally comes into equilibrium with the capillary pressure. In the injured vessels, however, the cells become concentrated into a solid cylinder, indicating the loss of practically all the fluid in the capillary and therefore of the plasma proteins.

That the permeability to colloids is increased is indicated by the fact that the straight line no longer crosses the line of zero filtration at 11.5 cm. as in the normal vessel, but at some pressure well below 5 cm. With considerably more difficulty the mesenteric vessels have been killed with 1:10,000 mercuric chloride solution. When, after this treatment, the rates of filtration are observed just as stasis is appearing, they fall upon the same line as those for 10 per cent alcohol, indicating that killing with these two substances produces essentially the same degree of increase in permeability.

IV. Discussion. The average pressure in the arteriolar capillaries of the frog's mesentery is 14.5 cm. and in the venous capillaries 10.0 cm. water. The direct measurement of fluid movement indicates that at pressures above 11.5 cm. the passage is outward and below that point inward. This relationship supports the theory of Starling that in general the direction of fluid movement is outward toward the tissues in the arteriolar end of the capillary network, and inward toward the blood in the venous portion.

White (1924) using collodion membranes as osmometers has placed the osmotic pressure of the plasma proteins for frog's blood between 10 and 12 cm. of water. The experiments here described, in a sense, make of each capillary an osmometer in which the osmotic pressure of the imprisoned blood appears to have an average value of 11.5 cm. It seems that for the mesenteric capillaries qualitative experiments with dyes, quantitative determinations of fluid movement, and the determinations of osmotic pressure all indicate the existence of a balance between capillary pressure and the osmotic pressure of the plasma proteins.

Another view of capillary permeability, in many ways opposed to that just discussed, must be considered. Krogh and Harrop (1921) have found that with the dilatation produced by the narcotic urethane in concentrated solution the permeability of the capillary wall increases to such a degree that plasma with its colloids drains off completely, leaving the corpuscles tightly packed together within the vessel. Capillary pressure was not measured but was inferred to undergo no change. Krogh (1922) concludes that: "No dilatation of capillaries involving mechanical stretching of the endothelium can take place without being accompanied by an increase in permeability, an increase which runs, on the whole, parallel to the degree of dilatation and which allows all the normal plasma colloids to filter off rapidly when the capillaries are strongly dilated." Other experiments led to the further generalization that "there is in almost all tissues an excess of the effective osmotic pressure over capillary blood pressure."

In the first group of experiments, however, it has been shown that there is no evidence of an increase in permeability with increasing diameter. On the contrary, capillary pressure measurements explain satisfactorily the variation in the rate of passage in different vessels. A study of the stasis produced by urethane (Landis, 1927b) has made it appear that the effect of this drug is due to injury, and is comparable to the increase of permeability obtained above by killing the endothelium, where also the wall became permeable to colloids. Finally there seems to be some indication that in the vessels studied there is a balance between average capillary pressure and colloid osmotic pressure. The evidence at hand indicates that dilatation does not increase the permeability of the capillary wall directly, though it may appear to do so in active hyperemia where there is a concomitant rise of capillary pressure.

Hirschfelder (1924) found also that in the edema produced by mustard oil vaso-dilatation increases the edema only when it also raises capillary pressure. Thus sodium nitrite locally increases the inflammatory effect of the irritant, but intravenous injection so lowers systemic blood pressure that the capillary pressure is insufficient to produce edema. Epinephrin inhibits the development of edema on the treated side for about two hours, at the end of which time the vaso-constriction due to the drug disappears. The lessened edema due to both this drug and to cocain are ascribed to a diminution of filtration pressure.

The importance of capillary pressure is noted also by Tainter and Hanzlik (1924) in their study of paraphenylenediamine edema. Loss of fluid could be prevented by a marked fall in systemic blood pressure and also by the administration of epinephrin. The latter raises systemic blood pressure but both procedures have the same effect on capillary pressure. Injection of pituitrin, which resembles epinephrin in its effect upon blood pressure, has been shown by Bayley, Davis, Whitman and Scott

(1925) to diminish the volume of lymph collected from the thoracic duct, and to delay the exit of physiologic salt solution from the blood stream. These observations emphasize the fact that it is not sufficient in studies of capillary permeability to record blood pressure and—as is often done—to assume that its changes indicate fluctuations of capillary pressure, since the two may vary independently and often in opposite directions.

Studies of capillary permeability are made difficult by the constantly varying diameter, pressure and flow in the vessels which form the capillary network. The number of variables can be reduced and the separate factors better controlled when single capillaries are studied. The experiments here described, carried out on this principle, are believed to indicate in a quantitative manner the effect of changes in capillary pressure, as distinct from systemic pressure, upon the passage of fluid through the walls of the mesenteric capillaries in the frog. The relative magnitude of the effects observed, even in injured vessels, indicates that before attributing changes in the rate of fluid movement to a modification of the capillary wall itself, it is essential definitely to eliminate change in capillary pressure as a possible factor.

SUMMARY

1. Micro-injection of dye solutions into single capillaries in the frog's mesentery indicates that the walls of dilated capillaries are not more permeable than those of constricted vessels.

2. The rate of passage of a dye solution through the capillary wall appears to depend upon the level of capillary pressure, not upon capillary diameter.

3. At any given pressure various dye solutions differ markedly in the time required for visible passage, due probably to their different colloidal properties. So far as permeability to dyes is concerned comparison of capillaries made of collodion to the mesenteric capillaries is not justified.

4. Vital red HR introduced into the ventricular blood passes immediately through the walls of those capillaries whose pressure is above 14 cm. water, but is retained for some minutes at pressures below 11 cm.

5. By direct measurement the rate of fluid movement through the capillary wall was found to be directly proportional to the difference between capillary pressure and the osmotic pressure of the plasma proteins, amounting to approximately 0.03 cubic micra per square micron of capillary wall per second for a difference of 5 cm. water pressure.

6. The osmotic pressure of the plasma proteins against their natural membrane as indicated by this method is 11.5 cm. water. When capillary pressure exceeds this value, filtration occurs, when it falls below it absorption results.

7. Capillaries injured by alcohol and mercuric chloride appear to be

permeable to the plasma colloids and approximately 7 times more permeable to fluid than the normal capillary wall.

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THE BILIRUBIN CONTENT OF THE BLOOD FOLLOWING INJECTIONS OF CHLOROPHYLL

JESSE L. BOLLMAN, CHARLES SHEARD AND FRANK C. MANN

From the Division of Experimental Surgery and Pathology, and Section on Physics, The Mayo Foundation, Rochester, Minnesota

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The chemical similarity between chlorophyll and hematin has frequently been mentioned in discussions of the source of bile pigment. It has been definitely established that hemoglobin is converted to bilirubin in the body, and the suggestion has been made that perhaps chlorophyll, or at least the pyrrol portion of the chlorophyll molecule, might also be converted into bilirubin. The importance of this possibility seemed to warrant its investigation, and although our results appeared to demonstrate that chlorophyll does not form bilirubin, a brief note of this fact seemed needed.

The methods employed in this study were similar to those which were used in the demonstration of the formation of bile pigment from hemoglobin (Mann, Sheard, Bollman and Baldes, 1926). The essential feature of the experiments consisted in obtaining by the spectrophotometric method of determination of bilirubin (Sheard, Baldes, Mann and Bollman, 1926) the rates at which the bilirubin content of the blood increased following ligation of the common bile duct and extirpation of the gall bladder in dogs. After sufficient time had elapsed to prove that this rate was uniform, an aqueous solution of chlorophyll was injected intravenously and the subsequent changes in the bilirubin content of the blood were studied at intervals. Just prior to the conclusion of each experiment laked blood was injected intravenously and specimens of blood were withdrawn after an interval sufficient to demonstrate the increased rate of bilirubin formation due to the injection of hemoglobin.

The results of this procedure were quite uniform in that no alteration in the rate of bilirubin formation occurred following the intravenous injection of chlorophyll. The injection of laked blood demonstrated an increased rate of formation of bilirubin, and numerous control experiments demonstrated that the development of bilirubinemia following ligation of the common bile duct and extirpation of the gall bladder, proceeded at a uniform rate, and that any alteration of this rate could be detected by the methods employed. From these experiments we were certain that bilirubin was not formed from chlorophyll under conditions which allowed the formation of bilirubin from hemoglobin.

The herbivorae excrete chlorophyll in the bile during the times when their diet is rich in chlorophyll. However, the sheep offers definite proof that chlorophyll may be altered in the body, since this animal elaborates a greenish pigment, cholehematin, when it is on green forage (Broun, McMaster and Rous, 1923). This pigment is also excreted in the bile, and is absent from the bile when the animal is on a diet containing little chlorophyll. When cholehematin is fed to dogs it is excreted as such in the bile. There seems to be little evidence that chlorophyll is converted to bilirubin in the animal, whether it is administered in the diet or whether it is injected intravenously.

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CARBON DIOXIDE AS A NARCOTIC AGENT

II. The Effect of Carbon Dioxide upon the Contraction of Striated Muscle of the Frog

CHARLOTTE HAYWOOD[1]

From the Department of Physiology, University of Pennsylvania

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The extensive and reversible inhibition of activity which carbon dioxide specifically produces in cells such as those of the developing echinoderm egg (Smith and Clowes, 1924; Haywood, 1927) suggests an inquiry as to its effectiveness upon striated muscle, which normally functions in the presence of more or less acid. The previous work in this field by Bottazzi (1916 a, b,) and others has been concerned with relatively large muscles, and under conditions where nervous elements might be present, and the resulting picture is therefore somewhat confused. The present investigation has as its object the further study of the action of carbon dioxide on striated muscle with the use of material believed to be somewhat more favorable than that previously employed. In particular, the attempt has been made to work with portions of muscular tissue so small and so thin as to favor the most rapid and complete exchange of gases possible with the surrounding solution and to use a method of recording contractions more delicate than that of the ordinary smoked drum. After trials of various methods it has been found possible to employ pieces of muscle about 6 mm. by 3 or 4 mm. in length and width respectively and 0.15 to 0.20 mm. in thickness and to measure contractions of 0.005 mm. The results here reported have been obtained under these conditions with the further precaution, usually neglected in the past, of the elimination of possible nervous factors by curarization.

Method. Electrodes, somewhat resembling those devised by Pratt (1917a) in their general arrangement, were constructed from two glass tubes, concentrically placed, and filled with a salt agar jelly into which dipped copper wires (fig. 1). The tubes were sealed into a hole in a flat block of paraffin (the electrode chamber) in such a way that their free ends partially formed the floor of a circular depression on the upper surface of the electrode chamber, which could be placed upon the stage of a

[1] Holder of the 1886 Fellowship of Mount Holyoke College (1926–1927) and of the Pepper Fellowship of the University of Pennsylvania (1926–1927).

microscope. The wires from the electrodes formed part of a secondary circuit, while the primary circuit consisted of a storage battery, a mercury key, and an induction coil arranged for repeated stimulation of a strength sufficient to evoke maximal contraction. In the later experiments, the mercury key was closed automatically by the pressure of a strip of spring steel upon which impinged an electrically driven cam. This method gave stimulations ranging in duration from 0.30 to 0.45 second and consisting of about sixteen to twenty-four individual stimuli, although during the course of any one experiment this entire range was probably never encountered. Stimuli of this short duration were ordinarily given at one minute intervals, a sufficiently generous recovery period to permit, in most cases, contractions of uniform amplitude.

Both the gastrocnemius and sartorius muscles of the frog were considered too large and thick to be useful for the problem at hand. However, the cutaneus pectoris muscle (Gaupp, 1896) of small specimens of *Rana pipiens* and of young *Rana catesbiana* was found to be admirably suited to the purposes of the work since the muscle is sufficiently small and thin to permit a rapid rate of diffusion, its fibers are parallel, and its attachments provide a convenient and firm point of support. After the muscle had been dissected out, as much of its surrounding membrane was removed as was easily possible, and for greater convenience the most lateral portion (usually about one-third or one-quarter) of the muscle was usually cut away. By means of fine needles the muscle could be held firmly in place in the electrode chamber containing Ringer's solution and its almost isometric contractions studied. Contraction was measured according to the method of Pratt (1917b) by observing with an ocular micrometer the excursions of a charcoal particle adherent to the muscle; in order to secure the maximum amplitude of movement, the particle was usually placed near one end of the muscle or upon the skin to which the muscle was attached. The depth of solution above the muscle was controlled by means of the focus of the microscope.

The Ringer's solution employed for most of the experiments was of the usual formula (Bayliss, 1924) except that the NaH_2PO_4 and glucose were omitted. The pH was lowered to 7.1 or 7.2 by adding a small amount of expired air. For larger amounts of CO_2, gas was added from a Kipp generator. The pH values of the solutions used were determined colorimetrically with brom cresol green, brom cresol purple, brom thymol blue, and phenol red, and are given without correction for the salt error.

The procedure of an experiment consisted first, of obtaining a series of contractions in ordinary Ringer's solution as a control, following which Ringer's solution containing CO_2 was pipetted on the muscle. Consistent results were obtained by frequently renewing the CO_2-containing solution in large excess between contractions. After the desired length of exposure

to CO_2, during which time the usual rhythm of stimulation was given, recovery in ordinary Ringer's solution was similarly studied. Since different muscles could not well be compared, a series of experiments was made on each muscle, using the same marker throughout. A graphic representation of contraction has been made by erecting upon an arbitrary base-line a series of ordinates which show the relative amplitudes of a series of individual contractions, the distance between successive ordinates corresponding to the time interval between successive stimulations.

RESULTS. The general depressant effect of CO_2 upon cell activity manifests itself, in the case of skeletal muscle, by a reduction in the height of contraction which is both immediate and pronounced. The regularity in the amplitude of contraction which is present when the muscle is stimulated in ordinary Ringer's solution at once disappears when Ringer's solution saturated with CO_2 (pH 5.2) is substituted. Even after one minute of exposure a significant reduction in contraction can ordinarily be observed

TABLE I

The effect of Ringer's solution, saturated with CO_2, in reducing contraction.

	NUMBER OF PREPARATIONS REPRESENTED	CONTRACTIONS AS PER CENT OF NORMAL CONTRACTION	
		After 1 minute of exposure	After 5 minutes of exposure
Range of values	27	95.5–42.2	57.7–3.8
Mean values	27	79.5	25.6
Modes	27	81.8	26.3

(Note: One preparation which showed only 10.5 per cent of contraction after one minute has not been included here.)

and by the end of five minutes the effect is very pronounced (fig. 3: *A*, *B*, and *E*). The extent of the diminution in contraction is given in table 1, which averages the mean values observed for each of twenty-seven different muscle preparations after one minute and five minutes of exposure. The average effect of CO_2-saturated Ringer's solution is thus to reduce contraction, even after only one minute, to about 80 per cent of its original value, and after five minutes, to about 25 per cent of the normal.

With longer exposures contraction continues to diminish, although at a decreasing rate. It is very nearly abolished by the end of ten or fifteen minutes, although a longer time is usually required before the response to stimulation entirely ceases. In a typical experiment it was found that the loss of contraction was almost complete by the end of ten minutes, but another ten minutes elapsed before stimulation absolutely failed to evoke a response. Even then, complete inhibition was not well maintained, and the very slight contractions which were later evoked from time to time seem

to indicate that the effect was not a progressive, highly toxic one, but rather a narcosis which was scarcely deeper than sufficed to stop contraction.

Although the tardiness with which this final reduction appears can very possibly be explained, in part, by small losses of CO_2, due to the method used, yet inspection of the contour of the record indicates it to be an exponential relation of the "die-away" type. This is shown by the fact that an approximately straight line is obtained by plotting the logarithms

Fig. 1. Diagram of vertical section of electrodes and electrode chamber. × 0.45. *D* = depression in electrode chamber. *X* = inner electrode. *Y* = outer electrode. Both *X* and *Y* are filled with salt agar jelly. *R* = rubber stopper holding *X* in place. *B* = stopper plugging *X*. *WX* and *WY* = wires emerging from *X* and *Y* respectively. *S* = microscope stage.

Fig. 2. The reduction of contraction of frog skeletal muscle at the end of five minutes' exposure to Ringer's solution containing varying amounts of carbon dioxide. Abscissa = CO_2 expressed as per cent of saturation value. Ordinate = Amplitude of contraction expressed as per cent of normal amplitude.

PERCENTAGE SATURATION	APPROXIMATE pH
100	5.2
80	5.3
60	5.4–5.5
50	5.6
25	6.1–6.2
$12\frac{1}{2}$	6.6

Temperatures: ● (Dots), 22° − 23.4°. × (crosses), 23.7° − 24°. ○ (circles), 24.2° − 24.8°. △ (triangles), 24.7° − 25.2°. □ (squares), 20.8° − 21°.

Fig. 3. The relative effectiveness of carbon dioxide, oxygen deficiency, and lowered pH in diminishing the amplitude of contraction of frog skeletal muscle. Light lines = contractions in ordinary Ringer's solution. Heavy lines = contractions in: *A*, *B*, *E*, CO_2-saturated Ringer's solution; *C*, nitrogen-saturated Ringer's solution; *D*, Ringer's solution acidified with HCl, giving a pH of 4.4. Temperature, 23°−24.1°.

Fig. 4. The relative effectiveness of CO_2-saturated solutions of varying $NaHCO_3$ content in diminishing the contractions of frog skeletal muscle. Changes in initial tension as well as in amplitude of contraction are shown. Light lines = contractions in ordinary Ringer's solution, M/420 with respect to $NaHCO_3$. Heavy lines = contractions in CO_2-saturated Ringer's solution, M/420 with respect to $NaHCO_3$ (pH 5.2−5.3). Dotted lines = contractions in CO_2-saturated solutions high in $NaHCO_3$. *X* = M/40 with respect to $NaHCO_3$ (pH 6.2). *Y* = M/15 with respect to $NaHCO_3$ (pH 6.6). Temperature, 20.2°−22.3°.

Fig. 5. The relative effectiveness of CO_2-saturated solutions of varying $NaHCO_3$ content in diminishing the contractions of frog skeletal muscle. Changes in initial tension as well as in amplitude of contraction are shown. Light lines = contractions in ordinary Ringer's solution. Heavy lines = contractions in CO_2-saturated Ringer's solution, M/420 with respect to $NaHCO_3$ (pH 5.2). Dotted lines = contractions in a CO_2-saturated solution, M/15 with respect to $NaHCO_3$ (pH 6.6). Temperature, 20.2°−22.2°.

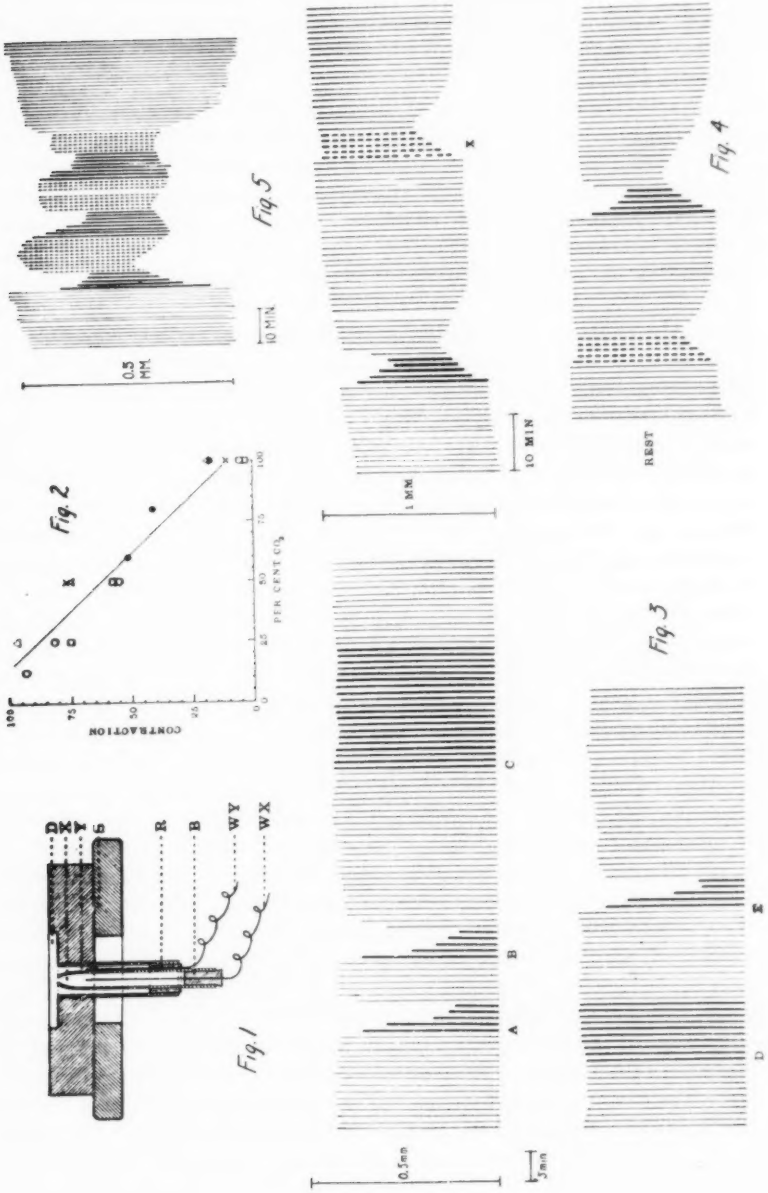
Fig. 1
WX
WY
B
R
S
Y
X
Fig. 2
CONTRACTION
PER CENT CO_2
Fig. 5
10 MIN.
0.5 MM.
Fig. 4
REST
10 MIN
1 MM
Fig. 3
A
B
C
D
E
0.5mm
3mm

of the height of contraction against the time. The slope of this line is such as to indicate that during the first six or eight minutes any given contraction is, on the average, approximately 0.8 as great as the one preceding it by one minute.

The action of concentrations of carbon dioxide below saturation value is less striking, but significant, and is usually manifested promptly. In figure 2 are charted five experiments which consist of two or more exposures, of five minutes each, to CO_2 of different concentrations, each exposure being followed by recovery in ordinary Ringer's solution. In two cases, Ringer's solution 25 per cent saturated with CO_2 (pH 6.1–6.2) was found to diminish contraction appreciably, and the intermediate values between this one and complete saturation indicate that the reduction of contraction is roughly proportional to the amount of CO_2 present.

As an indication of a narcotic rather than of a toxic action, the reversibility of the effects produced by carbon dioxide is of importance. The mere substitution of ordinary Ringer's solution for that containing CO_2 results invariably in some degree of recovery, notwithstanding the fact that no break has occurred in the rhythm of stimulation to allow a longer interval of rest. In general, the time required for recovery tends to vary inversely with the concentration of CO_2; for example, in one experiment recovery from practically 100 per cent saturated Ringer's solution required twenty-one minutes, while after an exposure of equal duration to 50 per cent CO_2 recovery was complete in thirteen minutes. Although the various muscle preparations have been found to show wide differences in their ease of recovery, yet after an exposure of five or ten minutes to a Ringer's solution saturated with CO_2 (pH 5.2) the effects of the gas can usually be completely reversed. The first minute after exposure is always found to give a conspicuous return toward the original amplitude of contraction. Complete recovery usually follows more gradually, but, once reached, is ordinarily well maintained. In general the same picture tends to hold true for somewhat longer exposures, such as those of twenty or thirty minutes. In such cases, however, the first minute following exposure gives a less conspicuous return of contraction, and subsequent recovery tends to be incomplete and less rapid. Recovery, even under these conditions, may be extensive; in one experiment an exposure of thirty minutes, which had practically abolished contraction for twenty-four minutes of that time, was followed by a return of contraction to 93 per cent of its original value; after the muscle was allowed to rest, without stimulation and in the absence of CO_2, for thirty-seven minutes, during which time recovery became practically complete, a further exposure of forty minutes was imposed and, in spite of the fact that stimulation evoked but a slight response during thirty-five minutes of this time, a recovery of 87 per cent was finally obtained. Even very long exposures to CO_2 permit some de-

gree of recovery. During one exposure of one and one-half hours there was absolutely no response to stimulation for the last thirty-eight minutes, and yet, after the substitution of ordinary Ringer's solution, a very slight contraction was evoked in two minutes, and by the end of two hours a 40 per cent recovery had been obtained.

In the experiments so far discussed, only the total contraction of the muscle has been measured. It is possible, however, by a slightly different method of graphic representation to take into account the initial tension of the muscle as well. In such cases the electrode chamber was held immovable during the course of an experiment, and both the excursion of the charcoal marker and its point of departure were determined with reference always to some one point on the micrometer scale; a corresponding plotting of the amplitude of contraction with reference to this same point thus serves to represent the changes in initial tension, as with the usual smoked drum record, by a changing base-line. When changes in the initial tension are thus taken into consideration, an increased contracture is seen to occur during exposure to CO_2 (fig. 4). This finding confirms Bottazzi (1916a) in showing that contracture is to some extent responsible for the resulting diminution in contraction. It will be observed that not only is contracture slow in disappearing, but it may even continue to increase for two or three minutes after the removal of the muscle from the CO_2-containing solution. The latter fact makes it unlikely that the diminished amplitude observed during exposure might be the result of an increasing initial tension, since after exposure the amplitude of contraction immediately returns toward its original value at the same time that the initial tension is still showing a slight increase,—an observation which may possibly be of interest in relation to Bottazzi's view that tonic contraction is independent of clonic contraction.

A question of much importance is the manner in which carbon dioxide produces its narcotic effects. Lack of oxygen in CO_2-saturated solutions can readily be ruled out under the conditions prevailing in these experiments, since solutions saturated with nitrogen and administered in an exactly similar manner as were the CO_2-saturated solutions have very little effect (fig. 3*C*). This result was of course to be anticipated from the observation of Fletcher (1902) that muscle may continue to contract for a long time in the absence of oxygen. A second possible factor is connected with the chemical nature of carbon dioxide. Unbuffered solutions containing considerable quantities of this substance have a more or less strongly acid reaction, and even when buffered to the point of actual alkalinity they may nevertheless produce an intracellular acidity (Jacobs, 1920a, b). One possibility, therefore, is that a change in pH either of the cells or of their surroundings is concerned. That the pH, as such, of the surroundings is of little importance is indicated by the fact that a decided

lowering of the pH of the solution by a "non-penetrating" acid such as HCl is almost without effect. It will be seen from figure 3*D* that the entire absence of inhibitory effects during a ten minute exposure to a CO_2-free solution of a pH of 4.4 offers a striking contrast to the marked decrease in contraction produced by an exposure, half as long, to a CO_2-saturated solution of pH 5.2. In no case could the negative results obtained from lowered pH and oxygen deficiency be due to a gradual decrease of sensitiveness of the muscle, since in this and other similar experiments CO_2 was shown to exert its characteristic action at both the beginning and the end of the experiment.

The effects of carbon dioxide on the internal pH of the cells is probably of much more importance than is the pH of their surroundings. The quantitative experiments of Smith (1925, 1926) indicate, in the case of "penetrating" acids, the importance of the concentration not only of the undissociated acid, but of its salt as well. To determine whether the presence of bicarbonate in a CO_2-bicarbonate mixture affects skeletal muscle similarly, experiments of the following type were devised. A solution was made up resembling the Ringer's solution ordinarily used except that for some of the NaCl there was substituted an equimolecular amount of $NaHCO_3$. After saturation with CO_2, the resulting solution, as compared to a CO_2-saturated Ringer's solution, was of the same CO_2 content and of the same osmotic pressure, but differed as to pH and as to bicarbonate and chloride content. The results of a typical experiment, illustrated in figure 4, show the reduction of contraction in five minutes to be, in this case, the following:

SOLUTION	pH UPON SATURATION WITH CO_2	CONTRACTIONS AS PER CENT OF NORMAL
Ringer's	5.2–5.3	44.0
Ringer's; M/40 with respect to $NaHCO_3$	6.2	64.0
Ringer's; M/15 with respect to $NaHCO_3$	6.6	72.0
Ringer's	5.2–5.3	31.0

More striking results of the same kind were obtained when the experiment was somewhat varied, as in figure 5, where the substitution of Ringer's solution high in bicarbonate, but saturated with CO_2, was found to allow some degree of recovery. It will be seen that, after this incomplete recovery obtained in the bicarbonate solutions, a second administration of Ringer's solution, saturated with CO_2, caused contraction again to be reduced. It thus seems evident that in the case of skeletal muscle as well as cardiac muscle (Smith, 1926) diminution of contraction is not determined solely by the concentration of the free acid. The modification of the characteristic effects of carbon dioxide by the addition of bicarbonate suggests the probability that the narcotic action of the latter substance is

related in some way to its ability to alter the internal reaction of the cell.

SUMMARY

1. An electrode chamber has been designed which permits the medium surrounding a very small muscle to be changed frequently without material alteration of contact with the electrodes.

2. In Ringer's solution saturated with CO_2 (pH 5.2) the contraction of the cutaneus pectoris muscle of the frog is on an average reduced in one minute to 80 per cent of the original value and in five minutes to approximately 25 per cent. In ten or fifteen minutes contraction may be very nearly abolished. The relation of the heights of contraction at successive intervals of one minute is roughly an exponential one.

3. Complete recovery occurs after exposures of ten minutes or less to CO_2-saturated Ringer's solution; following exposures of twenty to forty minutes recovery tends to be incomplete yet still extensive, and an appreciable recovery has been obtained after an exposure of one and a half hour.

4. With amounts of CO_2 corresponding to less than 100 per cent saturation the reduction in contraction is proportionally less.

5. The effects produced by CO_2 cannot be imitated by lack of oxygen nor by a lowering of the pH of the surrounding solution by a non-penetrating acid such as HCl.

6. The addition of bicarbonate in increasing amounts to a CO_2-saturated solution does not abolish the typical CO_2 effects, but diminishes them considerably in a manner similar to that reported by Smith (1925, 1926) for *Echinarachnius* eggs and for the turtle auricle. This effect of bicarbonates suggests that the narcotic action of CO_2 may be associated in some way with its ability to produce intracellular acidity.

It is a pleasure to acknowledge my indebtedness to Dr. M. H. Jacobs, who has been most generous with helpful criticism and encouragement.

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STUDIES OF THE THYROID APPARATUS. L

Interpretative Generalizations from the Differential Development Observed in Conditions of Thyroid and Parathyroid Deficiency

FREDERICK S. HAMMETT

From The Wistar Institute of Anatomy and Biology, Philadelphia

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Having, in the preceding papers of this series, reported and analyzed the growth response of the individual organs of the albino rat to thyroid and parathyroid deficiency, it is now possible to survey the animal as a particulate whole and attempt an interpretation from the point of view of differential development. It is toward this that the investigation as a whole has been pointed.

The study of the growth in weight of the individual organ is but the necessary prerequisite to the broader question of the rôle of the thyroid apparatus, as a part of the incretory coördinating mechanism, in the determination of development. In view of the findings discussed in preceding papers, it is hardly necessary to point out that a specific as well as a general value attaches to the study of the growth reaction of the individual organ. A concentration of interest on the specific value alone, however, yields results which are of limited practical or theoretical applicability, in so far as developmental relations are concerned.

The aims of the problem resolve themselves into the obtaining of answers to the following questions:

Is distortion of differential development produced by the glandular deficiencies? If so, what is its essential basis? What is the nature of the distortion? What is the basis of the differential reaction? Is the distortion consistent in direction? If so, what is the new type of organism produced? What are the implications to be derived from this? And what do these presage?

There are several ways in which the raw data may be handled in order to yield a picture of the influence of thyroid and parathyroid deficiencies on differential development. In view of the fact that the percentage rate of growth of the tests in terms of that of their controls has hitherto afforded the bast basis of comparison in the growth studies, and since a process as well as its end result is still being studied, these values are serviceable here as there.

The results have been recorded, either numerically or by chart in preceding papers. To reproduce the charts as there given would make analysis difficult. I have, therefore, plotted the data in a form which not only saves space, but also allows visualization of the classification into which the material separates itself.

Since the present interest is in the possibility of a distortion of bodily proportions, the degree of growth reaction of the organs is naturally compared with that of the body as a whole. To facilitate this the body weight growth after thyroid and parathyroid removal at the stated ages is represented on the charts by the heavy black line, and that of organ weight by the fine lines of severally different constructions. The numbers at the left of the charts are indices of the percentage rate of growth of the tests in terms of that of their litter mate controls of the same sex and glandular deficiency.

The criterion by which the absence or presence of distortion of differential development is to be determined, is obviously the agreement or lack of agreement of organ value with body weight value in any given age series. If the two are alike no distortion obtained. If they differ in degree it means that the growth of the one has been more or less affected than the growth of the other, as the case may be, and hence the proportion of total body weight represented by the particular organ in question is a lesser or a greater value than that normally present. This follows from the fact that the method of computation brings the data to the common level which is necessary when comparisons of this type are to be made.

An inspection of the charts shows immediately that some organs, such as the brain, spinal cord and eyeballs are less retarded in growth than the body as a whole (chart 1). Others, such as the pancreas, kidneys and adrenals are more retarded (chart 4), while others such as the submaxillary in the parathyroidless groups, and the hypophysis in the thyroidless males, appear to have had their growth accelerated (charts 5 and 7). The conclusion is inescapable that a generalized distortion of differential development is produced by thyroid and parathyroid deficiency.

In the analysis of the rôle of the thyroid apparatus in growth (Hammett, 1926a), the thesis was developed that the growth retardation which obtains in conditions of both thyroid and parathyroid deficiency, is the result of an essential undernutrition, and not of any specific growth relation to glandular function as such. This interpretation is sustained by the data as a whole, the details of which and of the argument need not be repeated here. The question now arises as to whether the difference in degree of growth response exhibited (that is to say, the distortion of differential development), is an expression of a specific influence of glandular activity on the growth of the individual organ, or whether it is an expression of the difference in relation of the functional characteristics peculiar to the differ-

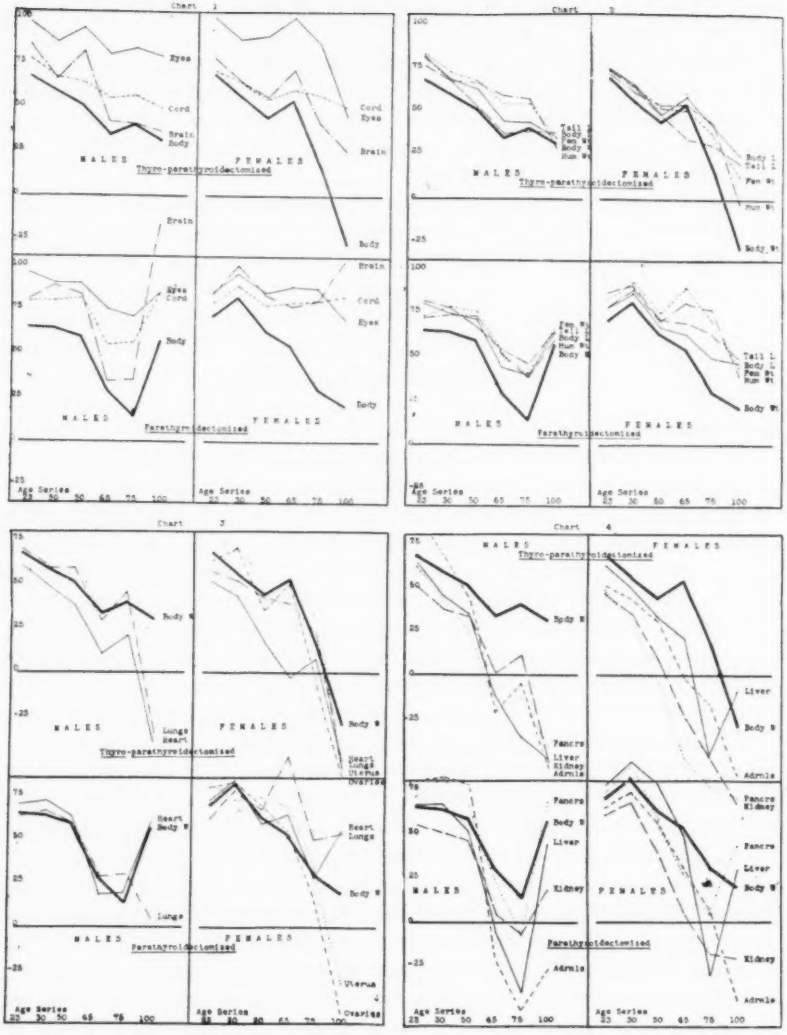

Chart 1. Compares the growth of the eyeballs, brain and spinal cord with that of the body after glandular removal at the stated ages.

Chart 2. Compares the growth of the humerus and femur in weight, and the tail and body in length with that of the body after glandular removal at the stated ages.

Chart 3. Compares the growth of the uterus and ovaries in the females, and the heart and lungs in both sexes with that of the body after glandular removal at the stated ages.

Chart 4. Compares the growth of the pancreas, liver, kidneys and adrenals with that of the body after glandular removal at the stated ages.

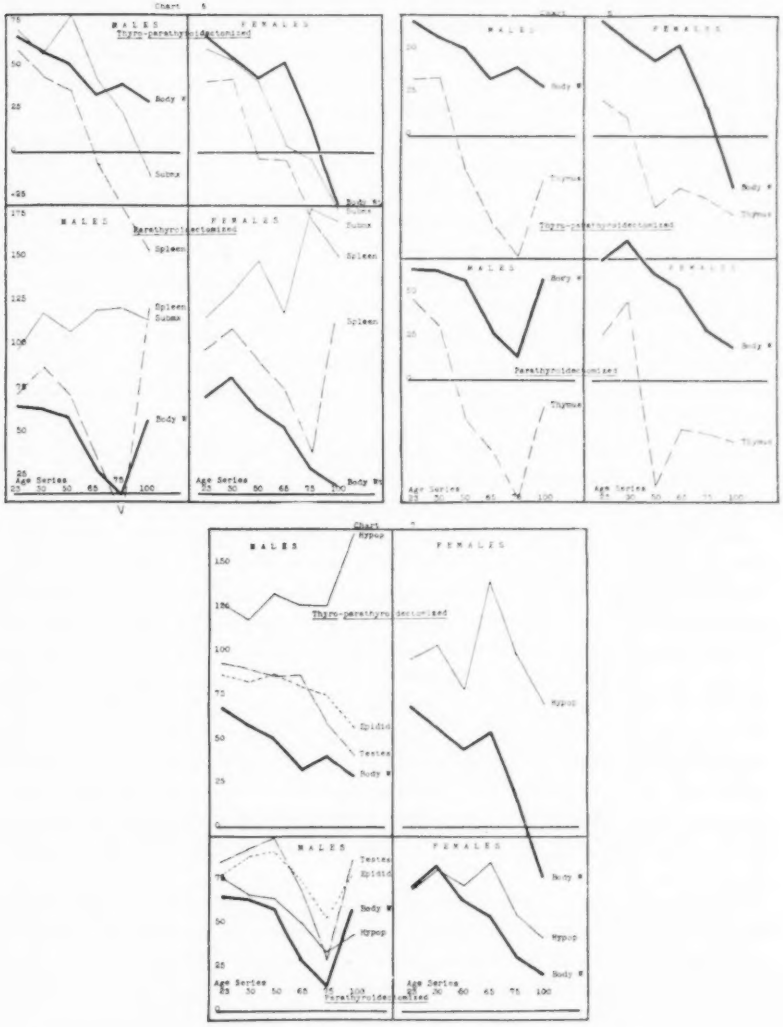

Chart 5. Compares the growth of the submaxillary glands and the spleen with that of the body after glandular removal at the stated ages.

Chart 6. Compares the growth of the thymus with that of the body after glandular removal at the stated ages.

Chart 7. Compares the growth of the testis and epididymis in the males and the hypophysis in both sexes with that of the body after glandular removal at the stated ages.

ent organs, to the lowered metabolic or nutritional level. This latter can justly be assumed to be a generalized reaction to the glandular deficiencies.

Now the charts show, with a few specific exceptions which are explained in the papers dealing with the separate organs, that the trend of direction of change in degree with change in age at time of glandular removal, is generally the same for the organs as for the body as a whole. This substantiates the thesis with respect to the rôle of the thyroid apparatus in growth, for it is obvious that the major factor in the growth retardation of the organs is their dependence on the growth processes of the body as a whole. Hence the conclusion is justified that the difference in degree of growth response of the several organs in conditions of thyroid and parathyroid deficiency is, in general, due to specific organ constitution and the relation of this to the metabolic or nutritional level, and not to any specific influence of glandular activity as such on the growth of the individual structure. Such is the essential basis of the distortion of differential development.

The nature of the distortion of differential development is ascertained by a study of the distribution of the organs with respect to the direction of their deviation in degree from the body as a whole.

From the charts it is seen that some give lines which are above, and some give lines which are below that of the body weight. The former are evidently more, the latter less resistant than the body as a whole to the growth inhibiting influences of the glandular deficiencies. Others, instead of having been retarded, seem to have been accelerated, for their lines lie above the 100 per cent or control growth mark. It would thus seem feasible to class the organs into Resistant, Sensitive and Accelerated groups. Such a classification, however, is merely a tabulation of the end-result. As will appear, certain organs which from their lines would be placed in either one or the other of the major groups, do not properly belong there. Hence I have placed them together with those of accelerated growth in what is conveniently designated as the Special group. The distribution is given in table 1.

Before going on to the interpretation of the basis of the differential reaction exhibited in the Resistant and the Sensitive groups, the bases of the specialization will be given.

First the organs of specific relation.

It is a matter of common observation that the hypophysis is an organ which reacts specifically to thyroid removal by hypertrophy (chart 7). No such growth relation has been demonstrated in these or any similar studies between the gland and the parathyroids. Hence, in the case of parathyroid deficiency, where specificity of growth relation is lacking, the hypophysis does not fall in the Special group, but in the group which is determined by the relation of its constitutional metabolism to the general

lowered nutritional level. Although hypophyseal hypertrophy is not a consistently occurring reaction to thyroid deficiency in the female (Hammett, 1926b), the character of the difference between its degree of reaction and that of the body as a whole justifies the assumption of a sex similarity in relationship, and hence its reaction in both female and male is classed as Special.

There is indubitably a specific reaction of the submaxillary gland to parathyroid deficiency (Hammett, 1927a). Hence the organ falls in the Special group. Since the lowering of the metabolic level occasioned by

TABLE I

Classification of the several structures according to their growth reaction to the glandular deficiencies relative to that of the body weight

RESISTANT		SENSITIVE		SPECIAL	
Chart number	Organ	Chart number	Organ	Chart number	Organ
1	Eyeballs	3	Lungs	7	Hypophysis (Thypars)
1	Spinal Cord	3	Heart	5	Submaxillaries (Parathys)
1	Brain	5	Submaxillaries (Thypars)	5	Spleen (Parathys)
2	Femur	4	Liver	7	Testis
2	Humerus	4	Kidneys	7	Epididymis
2	Tail Length	5	Spleen (Thypars)	6	Thymus
2	Body Length	3	Uterus (After puberty)		
7	Hypophysis (Parathys)	3	Ovaries (After puberty)		
3	Uterus (Before puberty)	4	Pancreas		
3	Ovaries (Before puberty)	4	Adrenals		

thyroid removal is of sufficient degree to largely prevent the appearance of the characteristic specific effects of parathyroid deficiency (Hammett, 1926a), submaxillary hypertrophy is not shown in the thyroidless rats (chart 5), and the organ takes the place determined by the relation of its type metabolism to the general disturbance.

Some justification is had for the belief that the spleen in parathyroid deficiency is affected adversely in a direction towards hypertrophy (Hammett, 1927b). Although the matter is by no means certain, it seems the part of better judgment to class the organ in the parathyroidless rats in the Special group (chart 5). For the reason given in the discussion of the

submaxillary, the spleen of the thyroidless animals falls in the group determined by the relation of its type metabolism to the general disturbance.

Now with regard to the exceptions.

As seen from chart 7, the testis and epididymis are less retarded than the body as a whole. From this it would be expected that these organs be placed in the Resistant group. But they differ from the rest of the body in a special way in so far as their growth is concerned by virtue of the functions they subserve. For the proportion of total growth attributable to growth by increase in cell number is obviously greater than that of the other organs. Moreover it appears as if growth by increase in cell number is more resistant than growth by increase in cell mass to the inhibiting influences of the experimental procedures (Hammett, 1926c). Hence, the testis and the epididymis belong and are placed in the Special rather than in the Resistant group.

The thymus is the most sensitive of all the organs to both types of glandular deficiency (chart 6). It is nevertheless put in the Special rather than the Sensitive group. I have done this because the interpretation of the differential sensitivity is based on general functional differences, and nothing is surely known of the functional or metabolic position of the thymus in bodily economy. Moreover, the organ is peculiarly sensitive to adverse conditions, and is unique in that the onset of its age involution generally appears long before that of the rest of the body. These facts deter me from including the thymus in the Sensitive group, notwithstanding its tremendous shrinkage under the experimental conditions (Hammett, 1926d). Until its functional and metabolic position is determined, its only legitimate location is in the Special group.

Two other apparent inconsistencies need explanation.

The characteristic functional metabolism of the ovary is obviously not attained until puberty. That of the uterus presumably lags behind until growth has brought about optimal structural development (Hammett, 1926c). Hence it is but natural that these two organs should shift their grouping once the functional metabolism is fully established (chart 4).

The lungs and heart tend to closely approximate the body weight with respect to the degree of growth distortion. Because of this and because the growth retardation of the parathyroidless rats was generally less than that of the thyroidless, these organs might be put in the Resistant group in the former and in the Sensitive group in the latter (chart 4). The absence of evidence of specificity of glandular function-organ growth relation; the consequent conclusion that the retardation is largely due to the lowered metabolic or nutritional level in both deficiencies; and the fact that where this was greatest the lungs and the heart tend to be more retarded than the body as a whole, lead me to class them with the relatively Sensitive organs.

Having cleared the ground of the Special cases, we are now ready to develop the answer to the question as to the basis of the differential reaction which leads to the distribution of the organs into Resistant and Sensitive groups.

Now each organ has its own characteristic rate of growth. On first thought it might be expected that this would be the chief factor in the determination of the differences in degree of growth retardation under the experimental conditions. But such is not the case. To demonstrate this point I have tabulated the various structures of the male rats thyro-

TABLE 2

The order of sequence of the several structures according to increasing growth capacity

TESTS	CONTROLS
Hypophysis	Epididymis
Eyeballs	Hypophysis
Epididymis	Femur weight
Testes	Submaxillaries
Humerus weight	Body weight
Spinal cord	Heart
Femur weight	Kidneys
Lungs	Humerus weight
Body weight	Lungs
Brain	Spleen
Submaxillaries	Testes
Heart	Pancreas
Kidneys	Eyeballs
Adrenals	Spinal cord
Pancreas	Liver
Spleen	Adrenals
Liver	Brain
Thymus	Femur length
Humerus length	Humerus length
Femur length	Tail length
Body length	Body length
Tail length	

parathyroidectomized at 75 days of age in the order of their increasing reaction to the glandular deficiency. These are given in the first column of table 2. The order of sequence is to be compared with that of the second column, which is the like record of the organs in the unoperated controls of the same sex and age series (Hammett, 1924).

It is at once evident that there is neither direct nor reciprocal concordance. This dislocation is exhibited in all the groups of all age series. It is therefore clear that inter-organ differences in inherent growth capacity under normal conditions are not indices of inter-organ differences in degree

of retardation under the experimental conditions. The explanation must be sought elsewhere.

Now the total energy expenditure of a given organ is divisible between that used for repair, that used for the work done in carrying out the function characteristic of the organ, and that used in growth or the incorporation of new material into the structure as a whole, thus adding to its weight which is here taken as the measure of growth. Of most significance for our present purpose is the ratio between the energy used for work and that used for growth. It is obvious that differences in specific functional characteristics determine differences in this ratio.

Now the group of Sensitive organs (table 1), the lungs, heart, submaxillary glands, liver, kidneys, spleen, uterus and ovaries after puberty, pancreas and adrenals, is a group the chief functional concern of which is to work over, elaborate, prepare, transport, or excrete materials for use by the rest of the body, mainly outside of the organ itself. They are the metabolic organs, as it were. It is just to assume that they have a higher work-growth ratio than do the organs comprising the Resistant group, the eyeballs, spinal cord, brain, femur, humerus, tail, back bone, hypophysis (?), and uterus and ovaries before puberty, in which no such generalized metabolic functional characteristic is exhibited.

It is a matter apparent to all that function continues when growth has been retarded or even stopped by non-lethal adverse conditions. Indeed it has been found in these experiments that function continues even when growth has been rendered retrogressive and the organs have lost instead of gained in weight. This fact justifies the generalization that, in conditions of essential undernutrition, work needs are taken care of at the expense of growth needs. Or, *function transcends increment.* This principle is at the bottom of the difference between the degree of organ growth retardation exhibited in the Resistant and the Sensitive groups.

The principle that function transcends increment requires that when energy expenditure is decreased because of decrease in energy supply, a greater than the usual proportion is used for function (work) and a less than usual proportion for growth. The natural consequence of this shift would be that in the organs of higher work-growth ratio, the relative increase in energy utilization for functional purposes would be greater than in the organs of lower work-growth ratio. The corollary of this is that, in the organs of higher work-growth ratio the relative decrease in energy utilization for growth purposes would be greater than in the organs of lower work-growth ratio. Hence the former would exhibit a greater growth retardation than the latter. It is this which is largely responsible for the separation of the organs into the Sensitive and Resistant groups.

Now it was stated earlier that the specific functional characteristics of an organ are determinants of its work-growth ratio. These also are asso-

ciated with the nature of the materials incorporated into the organ during growth. By them its compositional specificity is determined. The utilizability for metabolic purposes, and the resistance to disintegration of the specifically characterizing compounds are also factors in the growth response to adverse conditions. Their relative metabolic lability is of importance. For if they are metabolically labile they are more readily utilized under conditions of inadequate nutrition, than if they are relatively stable. That this differentiation is well taken is evidenced by the fact that the Sensitive organs, the organs which tend to lose weight under the growth-inhibiting influences of the glandular deficiencies, are organs which grow by increments of more metabolically labile materials since they are the organs of higher work-growth ratio, than do the Resistant organs of lower work-growth ratio. That is to say, the labile protoplasmic mass concerned in carrying out the specific function of the organ, is a larger proportion of the total mass in the organs of the Sensitive than in those of the Resistant group. In the latter there is the skeletal system (humerus, femur, tail and body length), characterized by deposits of relatively inert calcium salts: there is the nervous system (brain, spinal cord, hypophysis (?)), characterized by its relatively stable lipoids: there are the eyeballs, of which the humors of relatively inert metabolic participation are a large part: and finally there are the uterus and the ovaries growing by increments of materials of no general immediate functional utilizability. These then are the bases of the differential reaction to the glandular deficiencies.

An examination of the charts shows that there is a general consistency in the direction of deviation of organ growth development from that of the body as a whole. This holds regardless of the age at time of glandular removal, sex, or type of deficiency, save for the exceptions already discussed. It demonstrates that a new structural type of individual tends to be produced. The distribution of the organs as given in table 1 is an adequate picture of the type of shift in bodily proportions which obtains. The experimental procedures have yielded an animal in which the nervous skeletal systems are larger and the viscera of smaller proportions than usual of the total body mass.

In addition, data which have been derived from the values recorded in the preceding papers of this series, but which are not recorded here because of the desire to save space, show that the humerus and femur are longer as well as heavier than is usual in animals of the body weight recorded, and tend to be ever so slightly longer for the body length. This combined with the fact that the body length is less retarded than the body weight indicates that a more slender animal than usual has been produced.

It is a necessary as well as a legitimate part of scientific explanation to review the prospect presented from the eminence of experimental accomplishment. It is as essential to scientific progress to indulge in synthesiz-

ing speculation and outline the implications of the work done from the broader point of view, as it is to critically analyze and interpret the mechanisms leading to the detailed reactions observed. However, since this JOURNAL is not exactly suited to such a presentation, I have recorded my extrapolations from the data in another place (Hammett, 1927c).

The substance of the point of view there expressed is that "a fundamental distortion of the character of metabolism (i.e., of its inter-constituental relations) is potentially an adequate mechanism, whereby varieties within a species, species within a genus, and the inheritance of acquired characters are possibly producible."

Since it can justly be assumed that endocrine activity in general is largely concerned with the regulation of metabolism, it is not improbable that the incretory organs have been and indeed still are endogenous participants in the determination of evolutionary change in the organisms in which they are present.

SUMMARY AND CONCLUSIONS

Distortion of differential development follows both thyroid and parathyroid removal. With the exception of the hypophysis, the submaxillaries, and possibly the spleen, this is not due to any specific relation of glandular functions to organ growth, but to the general metabolic disturbance induced by the deficiencies. The growth of some organs is more resistant and that of others is more sensitive than that of the body as a whole. The basis of this differential reaction lies in the relation of the metabolic processes of the organs in question to the bodily economy as a whole. The distortion is consistent in direction and produces a distinctly modified type of structural organization. The broader aspects of this finding are referred to in their relation to evolutionary development.

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EFFECTS OF THERMAL AND CHEMICAL APPLICATIONS TO THE EXPOSED MEDULLA OF THE DOG[1]

E. S. GURDJIAN

From the Department of Anatomy, University of Michigan

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This work represents a study of thermal and chemical influences administered directly on the floor of the fourth ventricle. No attempt will be made at this time to review the anatomical and the physiological literature regarding this area and its relation to the physiology of respiration. A very good review of the literature on the mammalian respiratory center is given by Luciani (1911). For the purposes of this paper it is of interest to note that Gierke (1873, quoted by Luciani), as a result of experimental work concludes that the solitary bundle and the nucleus surrounding the same constitute the respiratory center. Gad and Marinesco (1893) arrived at the conclusion that the "noeud vital" of Florens does not constitute the respiratory center, but that the latter is located in the formatio reticularis deep in the substance of the medulla. According to these investigators the position of this center is different in different species of animals. Thus they locate it medial to the root of the hypoglossal nerve in the cat and lateral to the same in the dog. Electrical excitation of the formatio reticularis by means of fine varnished wires caused acceleration of the respiratory rhythm. Destruction of the formatio reticularis invariably meant arrest of respirations. The action of strong acids on the medulla (sulphuric, nitric) was studied by Brown-Sequard (1893). Their application to certain parts of the medulla caused arrest of respirations. This observer notes that most stimulation of the medulla is followed by an arrest of respirations rather than an increase in the rhythm of the same. Recently Lumsden (1922) claims to have located three respiratory centers in the cat, as a result of experimental work. The work of Allen (1927) on the visceral bulbospinal pathway shows that there are no direct bulbospinal tracts from the nucleus of the fasciculus solitarius and that most probably the neurones of the solitary nucleus influence the cells in the reticular formation of the medulla and that bulbospinal paths arise from the reticular formation

[1] This work was reported at the Nashville meeting of the American Association of Anatomists, April 1927, see bibliography.

and influence motor nuclei supplying muscles of respiration lower down in the spinal cord.

MATERIAL AND METHODS. Adult dogs were used. Morphine-urethane anesthesia was employed. Respirations and blood pressure were recorded in each case.

The medulla was exposed by electro-cautery. A midline incision is made through the skin. Then the muscles of the back of the neck are cut through in the midline as deep as the posterior arch of the atlas and the posterior margin of the foramen magnum, being careful not to puncture the posterior atlanto-occipital ligament at this time. Then the muscles are removed from the base of the occiput keeping in close proximity to the bone in so far as possible (this usually does away with all intramuscular bleeding). These muscles are now reflected laterally and the posterior arch of the atlas, the base of the occiput and the intervening posterior atlanto-occipital ligament exposed. During the whole process the head of the animal is kept flexed as far as possible, in order to bring about the widest separation between the atlas and the occipital bones. A transverse incision is made through the atlanto-occipital ligament. This incision should not be extended too far laterally because of the presence of small dural vessels in that region. With bone forceps the posterior margin of the foramen magnum is removed. In this process it is advisable to keep on the outside of the endosteal layer of the dura in order to minimize hemorrhage. Now the whole of the medulla and a portion of the cerebellum and the spinal cord are exposed.

EXPERIMENTAL DATA. Twelve successful experiments were performed.

The local application of lactic acid (n/10 and stronger solutions) for a period of three to five minutes produced no change in the respiratory frequency and the blood pressure.

The local application of carbon dioxide (both gaseous and dissolved in water) for a period of three to five minutes produced no change whatever in respirations and blood pressure (see fig. 2).

The effect of temperature (fig. 2) was studied by means of copper bars of proper size and shape to conduct heat or cold from an area of about 2 sq. mm. The temperature of the bars was raised by immersing them in water of a given temperature. Best results were obtained with temperature varying between 65° to 80°C. In one case the animal had complete respiratory arrest when copper at 95°C. was placed on area *C* in the medulla (refer to the discussion for the definition of the area *C*). By means of this technique we were able to locate on the dorsal aspect of the medulla an area heating of which was accompanied by an arrest of respirations and the removal of the bar was followed by a reappearance of the respiratory rhythm. This area is located just lateral and superior to the apex of the calamus scriptorius. It certainly corresponds to Florens'

"noeud vital" but includes more than a pin-point area. Microscopic preparations of the region are characteristic (fig. 4). Just inferior to the area *C* and surrounding the same is another area the heating of which is associated with less severe changes. A decrease in the frequency of the respiratory rhythm without a complete arrest of the same and irregularity

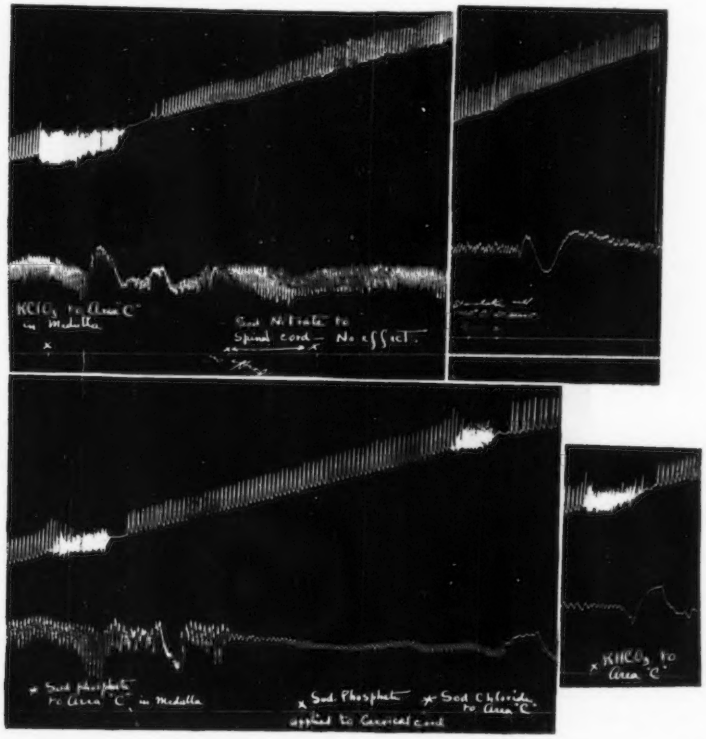
KClO3 to Area C in Medulla
Sod. Nitrate to Spinal Cord — No effect
x Sod. Phosphate to Area C in Medulla
x Sod. Phosphate applied to Cervical cord
x Sod. Chloride to Area C
x KHCO3 to Area C

Fig. 1. Curves illustrating the action of cold, potassium chlorate, sodium phosphate and potassium bicarbonate crystals on the respiratory rhythm when applied to the area *C* in the medulla. Note in particular the lack of any change in the respiratory frequency when some of these salts are applied to the spinal cord in the upper cervical region.

of the respiratory rhythm were the main changes noted. Stimulation of the rest of the dorsal aspect of the medulla was not associated with any respiratory change. We have termed the most susceptible area on the dorsal aspect of the medulla the area *C;* that surrounding the area *C* we have called the area *B*.

The application of cold to the dorsal aspect of the medulla in the form of ice or chilled copper bars did not give results in accord with those reported by Fredericq (1883) who found a slowing of the respiratory rhythm as a result of this technique (application of ice). In our experiments we noted that animals which were otherwise very sensitive to other types of stimulation either did not react to cold whatsoever or manifested

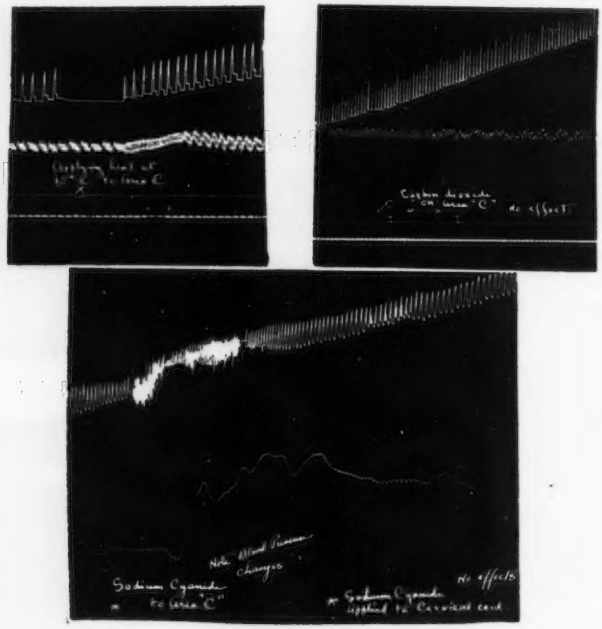

Fig. 2. Curves illustrating the effect of heat carbon dioxide and potassium cyanide on the respiratory rhythm, when applied to the area *C* in the medulla. Note in particular the lack of change in the respiratory rhythm when carbon dioxide is applied to the medulla in large quantities. Note also the tremendous increase in the blood pressure with cyanide application. When the latter is applied to the spinal cord no change in the respiratory rhythm is noted.

a slight increase in the blood pressure and a very slight irregularity of the respiratory rhythm. In more than one instance we applied ice to the medulla for a period of 3 to 10 minutes.

Unipolar and bipolar electrical stimulation was very difficult to use because of the associated movements of the body and neck. Irregularity in respirations and blood pressure were the chief changes noted. In this connection it is of interest to note that Ranson and Billingsley (1916) by

means of electrical stimulation of the floor of the fourth ventricle were able to locate two spots, a stimulation of one causing a depression of blood pressure, and that of the other causing a decrease in the blood pressure. Scott and Roberts confirmed the depressor point as described by Ranson and Billingsley.

The local application of strong salts such as sodium phosphate, sodium nitrate, sodium chloride, potassium chlorate crystals to the dorsal aspect of the exposed medulla in the region of the area *C* was followed by an increase in the respiratory rhythm and a slight increase in the blood pressure. The area *C* and its immediate vicinity are the only regions in the medulla which were responsive to these salts. The application of these salts to the spinal cord in the upper cervical region and to the cerebellum was not associated with changes comparable to the above mentioned (figs. 1, 2, 3).

The application of sodium cyanide crystals to the medulla in the region of the area *C* was associated with striking changes. There was a tremendous increase in the blood pressure accompanied by an increase in the respiratory rhythm (fig. 1). The application of sodium cyanide to the exposed spinal cord did not cause changes in respiration and blood pressure (fig. 1).

The effect of these different salts seems to be specific to the medulla. For example, no such changes were noted when the salts were applied to the spinal cord, cerebellum or to muscle tissue. We got the recorded changes only when the medulla in the region of the area *C* was the part stimulated. Are we dealing with an irritation of sensory fibers? If this were the case the application of these salts to the posterior funiculus of the spinal cord should also be followed by similar changes. This was not true, as shown in our experiments. One should keep in mind, however, the possibility of dealing with a special group of nerve fibers in the region of the area *C* and irritation of the same. Another important possibility is the stimulation of the grey matter under cover of the area *C* (see conclusions). The local action of these salts may be explained on the basis of an irritation due to 1, a dehydration of the region involved; 2, a direct action of the salt on the area; 3, a change in the acidity alkalinity ratio of the region. We are of the opinion that the first two factors are the more important ones operative in our experiments.

Conclusions. As the older writers had noted (Lagallois, Florens and others) there is an area in the region of the calamus scriptorius on the dorsal aspect of the medulla which if injured is followed by complete respiratory arrest. The area *C* on the dorsal aspect of the medulla as referred to throughout the present paper includes the "noeud vital" and the dorsal medullary surface surrounding the same to the extent of 3 or 4 mm. The heating of this particular region to about 75°C. was followed

by an arrest of respiration in the dog as shown in our experiments. Normal respirations ensued as soon as the modifying factor was removed. We know that the causative factor here is heat and not pressure, for the latter alone at the same area is unaccompanied by any noticeable change. The fact that there are no respiratory changes accompanying the irritation or the stimulation (as the case may be) of medullary tissue lower down would suggest that the area *C* may be a center having specific functions in the physiology of respiration. Examination of serial sections of the dog's brain stained with toluidine blue and silver methods reveals that the fasciculi gracilis and cuneatus and their respective nuclei extend as far

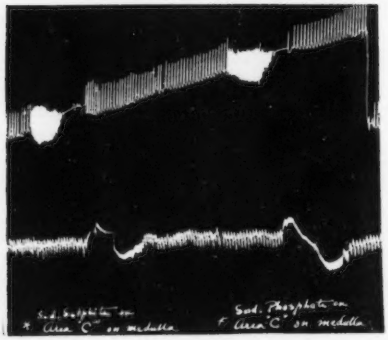

Fig. 3

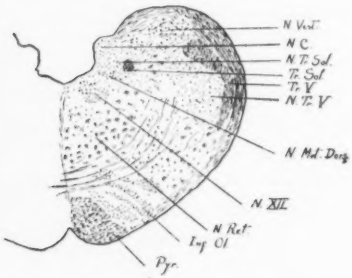

Fig. 4

Fig. 3. Curves illustrating the effects of sodium sulfite and sodium phosphate when applied to the area *C* on the dorsal aspect of the medulla.

Fig. 4. A cross section through the medulla of the dog passing just above the plane of the calamus scriptorius. *Inf. Ol.*, inferior olive; *N. C.*, the area *C*, the nuclear mass underlying the area *C* on the dorsal aspect of the medulla; *N. Mot. dors.*, dorsal motor nucleus of the tenth; *N. ret.*, reticular nucleus; *N. Tr. Sol.*, the nucleus of the solitary tract; *N. Tr. V.*, the descending nucleus of the trigeminal; *N. vest.*, vestibular nucleus; *N. XII*, nucleus of the twelfth nerve; *Pyr.*, pyramidal tract; *Tr. Sol.*, solitary tract; *Tr. V.*, descending tract of the trigeminal.

up as the lowermost plane of the calamus scriptorius. Farther cephalad at the most dorsomedial portion of the medulla one notes the presence of a nuclear mass made up of small and medium sized cells the whole complex protruding out in the form of an oval evagination well shown in our preparations (fig. 4). This nuclear mass underlies the area *C* and it is intimately associated with the nucleus of the fasciculus solitarius. In some forms investigators label it as the sensory nucleus of the tenth nerve (Winkler and Potter, 1914, for the cat; Craigie, 1925, for the rat). Our silver preparations show well-defined fibers interconnecting these areas. The cell type in this nuclear mass is essentially the same as in

the nucleus fasciculus solitarius. Through its connections with the nucleus fasciculus solitarius this mass in question is most probably in synaptic relations with the sensory fibers of the seventh, ninth and tenth cranial nerves. Through its connections with the reticular nucleus of the medulla (Allen, 1927) which sends reticulo-spinal fibers to the motor nuclei in the spinal cord (motor nuclei supplying the muscles of respiration), it completes the reflex arc between the sensory impulses coming from the respiratory organs (through the glossopharyngeal and the vagus nerves) and the motor impulses going to the muscles of respiration (through the reticulo-spinal paths which descend in the lateral and the anterior columns of the spinal cord). We are probably dealing with a correlation center between the sensory fibers coming from the respiratory organs and the motor fibers going to the same. A destruction of this nuclear mass by mechanical means is presumably accompanied by a shock of the same (due to a sudden stoppage of sensory or afferent impulses and the emission of efferent or motor impulses, whence respiratory arrest). The experiments of Marinesco and Gad suggest the same possibility. These observers were able to destroy this area by means of a method which permitted of a partial destruction of the tissue at each trial. They were thus able to destroy the whole area with no accompanying respiratory arrest (probably due to the fact that the destruction of the reflex arc was very gradual and that the neural tissue was given time to compensate). Clinical cases afford the same example in the human. Thus Brown-Sequard (1893) enumerates many instances of tumors in the region of the pons and the medulla, which cases at autopsy had shown complete atrophy and destruction of the "noeud vital" and the surrounding area. The premortem symptomatology was not characterized by extensive respiratory changes, as one should expect, which may be explained on the basis that the added injury at any given time (due to the increase in the size of the tumor) was so trivial that no shock or sudden derangement in the function of this correlation area could take place, and that the organism was able to accommodate itself to the new conditions (being given time to do so). Our own results also suggest the same point, i.e., that the heating of the area *C* to 75°C. does not constitute a sudden permanent damage to the region and consequently as soon as the modifying factor (the heated bar) is removed the respiratory rhythm (which had stopped under the influence of the mild damage?) reappears.

The salt action may be explained on the basis of a stimulation of the sensory fibers or a stimulation of the center where the sensory fibers end. The same result may be brought about by strong electrical stimulation of the central stump of the severed vagus.

It is noteworthy that carbonic and lactic acids, otherwise respiratory stimulants, were inactive when poured over the exposed medulla oblongata.

It is probably true that they are not absorbed fast enough to act as they normally do when introduced into the blood stream.

We have not been able to explain definitely the associated marked rise in blood pressure when crystals of sodium cyanide are placed on the dorsal aspect of the medulla in the region of the area *C*.

I should like here to express my gratitude and indebtedness to Professor Gesell for the supervision of this work and the placing at my disposal the facilities of the Physiological Laboratories, University of Michigan.

SUMMARY

Lactic acid and carbon dioxide did not alter the respiratory rhythm when placed in contact with the exposed dorsal aspect of the medulla oblongata.

Certain parts of the medulla were susceptible to the application of heat.

Strong salts when placed on the dorsal aspect of the medulla increased the respiratory rhythm. Sodium cyanide also caused a decided increase in blood pressure.

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EXPERIMENTAL BONE MARROW REACTIONS

IV. The Influence of Liver and Meat Diets on the Bone Marrow and the Regeneration of Red Blood Cells and Hemoglobin

GULLI LINDH MULLER

From the Thorndike Memorial Laboratory, Boston City Hospital

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In a series of investigations by Whipple, Hooper and Robscheit-Robbins on the effect of food on blood regeneration following single and multiple hemorrhages of different severity, it was found that liver exerted a beneficial effect on blood regeneration and especially on hemoglobin formation (1). Liver feeding in pernicious anemia was subsequently introduced by Minot and Murphy (2) and the excellent results obtained have stimulated interest in the effect of foods on the blood and blood-forming organs. This paper deals with the effect of liver and meat feeding on the hematopoietic organs of pigeons.

Pigeons were chosen as experimental animals because, by starvation, the bone marrow in the radius and ulna may be reduced to a state of comparative aplasia, the blood at the same time giving evidence of anemia. By this procedure it is thus possible to deplete the body of substances necessary for formation of blood cells.

In mammals starvation terminates fatally before any appreciable effect is observed on the blood and blood-forming organs.

Doan, Cunningham and Sabin (3) demonstrated that after starvation of pigeons, simple feeding will cause regeneration of the blood in a comparatively short time. The conditions thus seemed ideal for the study of the quantitative and qualitative regeneration of the blood when animals were fed with different foods. It was thought that by starting with the simplest conditions possible some lead might be obtained as to the specific effect of liver on the hematopoietic organs in pernicious anemia. No claim is made that results obtained with pigeons are applicable to man, although other investigators of food and its effects on the organism, including McCarrison (4), who worked with pigeons and monkeys, have found that, allowing for small differences, the fundamental nutritional processes in birds and mammals are the same.

Experimental methods. The pigeons were starved from seven to thir-

teen days, fresh water being given every day. They lost from 63 to 130 grams during this period. After the period of starvation they were fed with:

1. A mixed grain diet obtained in the market and used as stock pigeon feed (group I).

2. Broiled beef liver, medium done to rare, cut up in small pieces by hand to avoid the loss of juices and to obtain pieces which could be consumed without difficulty (group II).

3. Broiled beef muscle treated in the same way as the liver (group III).

A few animals were similarly fed without the initial period of starvation.

The pigeons ate eagerly as a rule, although one was occasionally lost because of complete refusal of food at the end of the starvation period. In such cases grain was refused as well as liver and meat. The weight, red blood cell count, and hemoglobin content of the peripheral blood were recorded at intervals. Blood was obtained from the vein on the wing by puncture. The hemoglobin was read in a Duboscq colorimeter against the Newcomer standard. At the end of the experiment liver, spleen and bone marrow from the radius and femur were fixed in Zenker's fluid, stained with watery eosin 5 per cent, and counterstained with methylene blue. Ammonium sulphide and potassium ferricyanide were used for the iron stain.

Experimental observations. In interpreting the data obtained two factors must be considered; namely, the replacement of the lost weight and the regeneration of the blood. The number of red blood cells in the normal pigeons used averaged about 4,000,000 and the hemoglobin 95 per cent. The number of red blood cells after the period of starvation varied from 2,300,000 to 4,300,000 and the hemoglobin from 46 to 91 per cent. The higher red blood cell counts and hemoglobin were obtained in animals starved for a short time, and were due probably to dehydration. The decrease of erythrocytes and hemoglobin as a rule was directly proportional to the loss of weight. In from two to four days after the beginning of the feeding red blood cells and hemoglobin fell considerably in all animals. This may have been due to a dilution of the blood as well as a destruction of erythrocytes without adequate replacement of lost cells.

After being fed the animals within each group showed a constant reaction with insignificant variations. The results obtained will therefore be given as the general reaction of the group rather than of the individual animal.

Group I: Mixed pigeon feed. Pigeons fed grains are taken as the standard. The initial weight was regained in from eleven to twenty-four days. As a rule erythrocytes and hemoglobin were regained in from thirteen to twenty-one days. In one animal losing 130 grams, the initial weight was

not regained in twenty-one days, but red blood cells and hemoglobin had reached normal values.

Group II: Liver diet. Pigeons fed liver regained their approximate initial weight in thirteen to twenty days. The highest number of red blood cells and hemoglobin was reached in about twelve days, ranging from 3,000,000 to 3,600,000 erythrocytes and 76 to 79 per cent hemoglobin. Then the red blood cells and the hemoglobin began to decline steadily and when the erythrocytes were approaching 2,000,000 cells and the hemoglobin 50 per cent, the appetite, good up to this time, began to fail. The animals were killed before any appreciable decline of weight had taken place, in most instances when they were within ten grams from the initial weight. As a rule the pigeons looked healthy at the end of the experiment.

Group III: Meat diet. In contrast to the pigeons fed liver, those on meat diet had an excellent appetite throughout, regained their initial weight, red blood cells and hemoglobin as rapidly and in some instances more rapidly than control pigeons fed with mixed grain. Their condition at the end of the experiment was as a rule excellent. The general course of individual animals within the three groups is illustrated in table 1.

At necropsy the most striking gross feature was the yellowish-orange tinge of the liver in the liver-fed animals in contrast to the deep brownish-red in the control pigeons. The consistency of the liver in the liver-fed pigeons seemed firmer than normal and apparently the size was increased. No constant gross change was observed in the spleen, an insignificant organ in the bird. The bone marrow of the radius was voluminous, and reddish-pink in contrast to the normal moderately fatty radial marrow. The femoral bone marrow of the normal pigeon is usually red while in liver-fed animals the marrow was pale red, voluminous and quite firm.

Microscopically the bone marrow of the pigeons starved until a loss of 100 to 130 grams weight had been obtained, showed essentially the same features as have been previously described (3), i.e., the bone marrow of the radius and ulna was practically aplastic with disappearance of the fat globules; the femoral bone marrow was hyperplastic. This hyperplasia was essentially leucocytic. The red blood cells present were of the late erythroblastic stage or mature forms. The structure of the femoral bone marrow in these starved animals showed very little or no arrangement of the cells and the globules in the fat cells were practically absent.

The marrow of grain-fed pigeons is taken as the normal standard, with and without a period of starvation. The radial and the femoral bone marrow were examined. The essential features as regards architecture and composition of cellular elements in the two situations seemed similar but the radial marrow was in all instances more fatty than the femoral in the center, with varying amounts of hyperplasia at the edges. In the three

groups of animals the reaction of all in each group was fairly constant. The general features of each group will be described:

1. Grain-fed pigeons: The bone marrow showed a definite pattern or arrangement of cells. The erythrocytic centers were well circumscribed,

TABLE 1

NUMBER	INITIAL WEIGHT	DAYS OF STARVATION	LOSS OF WEIGHT	R.B.C. AT END OF STARVATION	HGB. AT END OF STARVATION	FED NUMBER OF DAYS	FINAL WEIGHT	LOSS OR GAIN OF WEIGHT	FINAL NUMBER OF R.B.C.	FINAL PER CENT OF HGB.
Grain										
	grams		*grams*		*per cent*		*grams*	*grams*		
6	297	8	108	2,800,000	48	17	306	+9	4,450,000	92
7	339	9	130	2,330,000	53	21	294	−45	4,230,000	89
14	276	9	99	2,840,000	66	53	292	+16	4,030,000	97
15	391					29	416	+25	4,390,000	99
16	280					64	322	+42	4,640,000	92
Liver										
12	344	7	98	2,340,000	60	4	299	−45	1,940,000	54
44	323	13	108	2,990,000	68	5	271	−52	2,910,000	74
45	355	11	130	2,350,000	48	12	330	−25	2,880,000	59
5	299	8	103	2,360,000	46	17	290	−9	3,040,000	64
9	334	9	126	2,700,000	54	21	333	−1	2,440,000	51
19	290	9	84	3,440,000	88	27	275	−15	2,600,000	66
48	328	11	102	2,910,000	68	31	318	−10	1,980,000	48
27	290	8	75	3,380,000	79	32	285	−5	2,080,000	48
28	308	8	63	4,320,000	91	39	326	+18	2,580,000	61
24	223					49	217	−6	2,210,000	45
40	330					49	353	+23	2,600,000	59
Meat										
20	300	11	121	2,810,000	63	4	200	−100	1,690,000	50
8	312	11	110	2,770,000	63	21	352	+40	3,800,000	96
29	322	8	87	4,000,000	91	32	295	−27	3,930,000	92
31	292	8	80	3,350,000	79	39	291	− 1	3,640,000	89
18	294	9	97	3,530,000	80	53	320	+26	4,310,000	92
42	368					49	402	+34	4,450,000	103
26	240					54	272	+32	3,950,000	101

enclosed in what appeared to be endothelial confines. This is very striking in comparison with mammals. The shape of these centers varied with the section with many round or oval aggregations of cells or longitudinal strands. The latter oftentimes stretched across from the edges of the bone marrow section to practically the center (fig. 1). In a longitudinal

section of the bone marrow this corresponds to the arrangement of the blood vessels. These aggregations of cells consisted of megaloblasts, in many

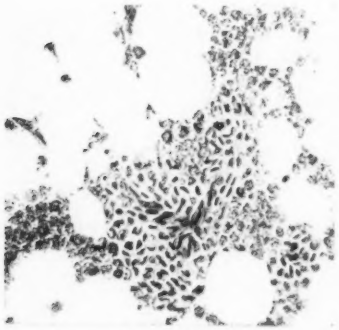

Fig. 1

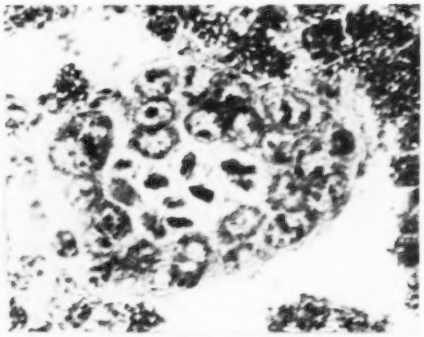

Fig. 2

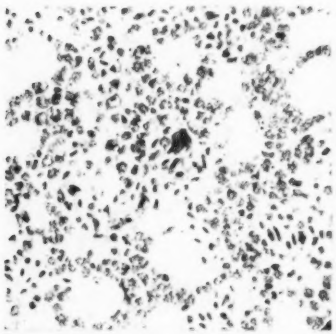

Fig. 3

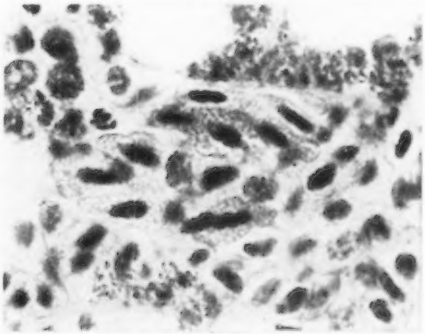

Fig. 4

Fig. 1. Pigeon 14. The general architecture and cellularity of the femoral bone marrow after 53 days of grain feeding. × 750.

Fig. 2. Higher magnification of erythrocytic center in pigeon 14. The circumscribed center with characteristic arrangements of cells is striking. The youngest cells are at the periphery, while the mature cells are in the center. × 1250.

Fig. 3. Pigeon 27. Architecture of bone marrow 32 days after liver feeding. Note the greater cellularity, and the small erythrocytic centers, composed mainly of almost mature red blood cells. × 750.

Fig. 4. Higher magnification of erythocytic center in pigeon 27. The absence of megaloblasts as contrasted with figure 2. is illustrated. × 1500.

cases, erythroblast and adult cells. The arrangement was definite and ought to be emphasized. At the periphery were the megaloblasts or the youngest cells, while in toward the center the cells graded off into hemo-

globin bearing adult red cells (fig. 2). Thus the pattern of the cells speaks for the endothelial intersinusoidal origin of red blood cells. The number of erythrocytes of all stages seemed to be about equal to leucocytes. This was verified by supravital counts of the bone marrow. An interesting feature of the normal pigeon bone marrow was the presence of a number of megaloblasts, a feature not encountered in mammals as represented by rabbits and man. It seems as if in pigeons the process of hematopoiesis began in an earlier stage in the erythrocytic series and stopped short at the mormoblastic stage. During the first two weeks after starvation hematopoiesis was active, the normal architecture was established, but even as late as on the fifty-third day of feeding megaloblasts seemed more abundant than in the non-starved animals.

2. In the meat-fed animals the microscopical findings of the bone marrow were essentially the same as in the grain-fed pigeons.

3. The radial and the femoral bone marrow of liver-fed pigeons were in all instances more hyperplastic (fig. 3). This was especially true of the radial marrow. At the various stages examined during the first three weeks of feeding following the starvation period several features stood out. The regeneration on the fourth and fifth days was greatly advanced and the bone marrow showed a great many megaloblasts. As far as can be judged from the material at hand the regeneration obtained was more than adequate and possibly superior to the regeneration obtained with meat or grain. On the twelfth and seventeenth days the activity of the erythrocytic centers had abated considerably and at the end of the third week the proportion of the cells of the erythrocytic series had diminished, the erythrocytic centers seemed shrunken and were composed mainly of late erythroblasts and adult red cells with few megaloblasts (fig. 4). The white blood cells were correspondingly increased as the marrows retained their hyperplastic appearance.

In summary, therefore, the outstanding features in the marrows of liver-fed pigeons after a period of starvation were adequate red blood cell formation during the first two weeks after feeding was begun, with subsequent depression of erythrocyte formation, and especially of the megaloblasts or the youngest forms.

The liver, in the liver-fed animals, showed extensive fatty deposits the nature of which has not yet been determined.

The blood serum of the liver-fed animals showed a marked milky opalescence. In meat-fed and grain-fed pigeons the serum was clear or very slightly cloudy as a rule. Food was withdrawn for twenty to twenty-four hours before the termination of the experiment so as to exclude alimentary lipemia. The serum was examined for cholesterol and lecithin. In grain-fed pigeons the cholesterol content in the serum, determined by Bloor's (5) method, varied between 335 to 380 milligrams per 100 cc.

of serum, in meat-fed animals between 382 to 765 mgm. per 100 cc. of serum, while in liver-fed animals between 735 mgm. to 2.083 grams, the lowest value determined being obtained on the fifth day after commencement of liver feeding. In animals killed at the end of the period of starvation, the cholesterol in the serum was invariably less than normal. No definite trend could be made out with regard to lecithin phosphorus, determined by Whitehorn's method (6). Variation within the normal limit was observed both in grain, meat and liver-fed pigeons.

Discussion. To summarize the results obtained one finds that mixed grain diet and beef muscle when fed to pigeons after a period of starvation produce practically an identical picture with regard to replacement of weight and adequate regeneration of red blood cells and hemoglobin. The bone marrows, similar in structure and cellularity, showed an abundance of erythrocyte formation and a great many megaloblasts, the earliest recognizable form of the erythrocyte series. In contrast to this, liver feeding produced definite physiological differences: i.e., an early adequate blood formation followed in the third week by a definite depression of red blood cell formation. This depression was evidenced by a fall in red cells and hemoglobin in the peripheral blood, a decrease of immature erythrocytes in the bone marrow, and especially a decrease of the number of megaloblasts. This diminution or suppression of cells does not involve the white blood cell series, the number of which was apparently increased as shown by the greater cellularity of the bone marrow and the supravital counts.

In attempting to explain the anemia produced by liver feeding, one may consider three hypotheses; namely, an increased blood destruction, an inadequacy in the liver diet of the substances necessary for blood formation, or the excess of some inhibitory substance in the presence of which blood regeneration and hemoglobin formation are suppressed or greatly diminished.

There are no evidences of an increased blood destruction. At the end of the period of starvation considerable iron was found in the "reticuloendothelial" system. As the blood picture improved after feeding this iron disappeared and at the end of the experiment comparatively little iron was found in the hematopoietic organs.

The inadequacy of liver as a complete food must be seriously considered. Both liver and meat may in some sense be considered as food foreign to pigeons, but the adequacy of meat from the point of view of blood formation when contrasted with the effects of liver feeding on the blood forming organs was striking. In nutritional studies liver has been claimed to be far superior in its protein to muscle meat (7), (8); the fat soluble vitamin A and water soluble vitamin B (8), (9), (10) and the antiscorbutic substance (10) are present in abundance. Muscle tissue is deficient in these vitamins

(8), (9), (10), (11). The glandular organs, however, are not superior to the muscle tissue in their mineral content (7). On the whole, liver, kidney and sweetbread are superior to any other animal tissue (7). The glandular structures are, like muscle, lacking in calcium (7), (10). The superiority of liver in hemoglobin formation as compared with meat has been demonstrated by Whipple and associates (1). If inadequacy, therefore, of the food supplied is to be considered, we would expect to find signs of this in the meat-fed group.

Finally, one may assume that the anemia produced is due to an excess of some substance in the liver diet which suppresses not only the red blood cell formation but also the formation of hemoglobin. The inhibition is primarily effective on the youngest cells, probably in the premegaloblastic stage, i.e., the endothelial cells. That megaloblasts are produced by the endothelium of the intersinusoidal capillaries becomes a conviction when studying bone marrow of pigeons at different periods of regeneration.

In this connection the results of Heaton (12) and Morrill (13) if verified are of interest. Heaton found that extracts of liver inhibited growth of fibroblasts in vitro, but epithelial cells were unaffected, while Morrill showed that regeneration of limbs was delayed and inadequate in liver-fed newts while regeneration proceeded normally in meat-fed animals.

The results obtained in the experiments here described may throw some light on the effect of liver feeding in pernicious anemia, and the remarkable results obtained. The bone marrow in pernicious anemia shows hyperactivity as evidenced by the great number of megaloblasts and frequent mitosis. The hyperplasia is extensive throughout. A very short time after liver feeding is begun, or a remission sets in due to any cause, the megaloblasts disappear, the bone marrow loses its hyperplastic structure, and the normal architecture and cellularity are restored. This has been shown to take place by Peabody (14) by the use of intravitam bone marrow punctures at various stages of remission. Concomitant with this the bilirubinemia, indicating a somewhat increased blood destruction or hyperactivity of the "reticulo-endothelial cells," the site of bile pigment formation, disappears. These features are most striking in the pernicious anemia remission.

The effect of liver feeding in pernicious anemia may be explained by the addition of some substance which causes maturation of the young erythrocytes. This opinion seems to be most prevalent at the present time. It does not, however, explain the rapid cessation of megaloblast formation, the prominent feature in this disease, nor the disappearance of the bilirubinemia.

The experiments described above suggest an hypothesis of an inhibitory substance in the liver checking the excessive fundamental stimulus to growth and suppressing the formation of megaloblasts at its earliest stage,

as well as the hyperactivity of bilirubin formation. A normal balance is thus restored between stimulating and inhibitory substances, which one must assume governs all cell growth. If this substance is in excess a depression of cell and hemoglobin formation occurs as seen in the experiments described above. This inhibitory effect is most evident in the megaloblastic stage of the erythrocytes, the normal source apparently of adult red blood cells in pigeons. Reversion to this source is seen in pernicious anemia, while normally red blood cell formation in man takes place from an erythroblastic or normoblastic stage.

As to the nature of this substance, no conclusions can be drawn from these experiments. There is a marked increase of cholesterol in liver-fed pigeons. Most investigators agree that there is some disturbance of lipoid metabolism in pernicious anemia and practically all agree that cholesterol is low in the serum. Evans (15) in his review of the possible etiology of pernicious anemia feels that the lipoids play an important part. Whether cholesterol, its esters or some other substance is the active principle in the suppression of blood formation in pigeons, and whether this substance is identical with the active principle in pernicious anemia, only the future will show.

CONCLUSIONS

1. The effect of grain, beef liver and beef meat on the blood regeneration of pigeons made anemic by simple starvation was studied.

a. Grain diet in form of pigeon feed caused regeneration of red blood cells and hemoglobin in less than three weeks.

b. Beef meat was as effective and in many instances more effective than grain.

c. Beef liver caused a rapid regeneration of red blood cells and hemoglobin up to about the twelfth day when erythrocytes averaged about 3,300,000 and hemoglobin 77 per cent (normal averages 4,000,000 red blood cells and 95 per cent hemoglobin). Then the red blood cells and the hemoglobin began to decline steadily.

2. The weight of all the animals was regained rapidly. The appetite was good throughout except in the liver-fed group in which it began fo fail when the red blood cells and hemoglobin had declined to about 50 per cent of normal. Pigeons in this group were killed before any appreciable loss of weight had taken place.

3. The bone marrow of the radius and ulna at the end of the starvation was aplastic.

4. The grain and meat fed pigeons showed essentially the same features in the bone marrow when normal red blood cells and hemoglobin values had been reached; namely, large islands of the erythrocytic cells composed of many megaloblasts at the periphery with more mature cells in the center.

5. The bone marrows of the liver fed animals up to the twelfth day were essentially the same as those of the grain and meat-fed pigeons. With the decline of red blood cells and hemoglobin in the peripheral blood the erythrocytic centers became smaller, the cells were more mature with the disappearance of the megaloblasts.

6. The cholesterol in the serum of the liver-fed pigeons was increased to five times its normal value. Lecithin phosphorus showed no abnormal trend.

7. The hypothesis, that the suppression of red blood cell and hemoglobin formation is due not to a lack of substances in the liver but to the excess of some inhibitory substance, is considered.

8. It is suggested that the factor of inhibition or suppression of blood formation in the megaloblastic stage may be the beneficial factor in the liver diet treatment in pernicious anemia.

I am indebted to Dr. Henry Jackson, Jr., for aid with the photomicrograph and it gives me great pleasure to acknowledge his valuable assistance.

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FACTORS INFLUENCING THE ABSORPTION OF WATER AND CHLORIDES FROM THE INTESTINE

JACOB RABINOVITCH

From the Department of Physiology, Washington University, St. Louis

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The mechanism by which the various forces concerned carry on the process of absorption from the intestine has long been a question of dispute. The literature of the subject has been reviewed by Goldschmidt (1921). It is of course well known that certain physical and chemical forces, for example, the osmotic pressure of the intestinal contents, the chemical nature of the solute, etc., will affect the absorption of both solvent and solute. The manner in which these forces work together to bring about the final results is, however, unknown. It is the purpose of this paper to report the results of some experiments designed to investigate the influence of some factors on intestinal absorption under as nearly as possible physiological conditions.

Effect of varying the osmotic pressure of the intestinal contents. In our first series of experiments, the concentration of sodium chloride in the solution introduced into the colon was varied from 0.1 per cent to 1.1 per cent and the absorption of water and chloride followed.

In this series the large intestine in dogs was used. A permanent colostomy was made and the animals allowed to survive. The proximal end of the colon was brought out through the abdominal wound and served for the introduction of fluids into the gut; the rectum was used to collect the fluid after it had stayed in the gut for a definite length of time. The abdominal wound healed completely within two weeks after the operation, and the following experiments were then made.

In these experiments determinations of the amount of fluid and chloride secreted by the experimental loop of gut were not made, and therefore the actual quantities of fluid and salt absorbed are not absolute. It will be noted, however, in table 1 that when using hypotonic sodium chloride solutions, the percentage of salt in the fluid recovered after it has been subjected to intestinal action for 30 minutes, is higher than the percentage of chloride introduced into the gut. Also when a 0.1 per cent sodium chloride solution is used, the quantity of chloride recovered from the gut is greater than the amount introduced. It appears from these figures that no salt is being absorbed from such dilute solutions of sodium chloride (i.e., 0.1

per cent) but chlorides are rather thrown into the gut contents, making the total chlorides recovered greater than that introduced. As will be shown later, in the case of the small intestine where estimations of chlorides in the secretions have also been made and taken into account, absorption of chlorides takes place even when such hypotonic salt solutions are used. Absorption of chlorides from 0.2 per cent solutions and stronger is apparent even when the chloride secreted is not taken into account; the extent of absorption of chloride increases with increasing concentrations of solutions introduced. It will also be noted that at a salt concentration somewhere between 0.5 and 0.6 per cent the percentage of chloride recovered from the

TABLE 1

Absorption of water and chlorides from the colon

DATE	CHLORIDE IN SOLUTION INTRODUCED	VOLUME INTRODUCED	VOLUME RECOVERED	VOLUME ABSORBED	WATER ABSORBED	NaCl INTRODUCED	CHLORIDE IN SOLUTION RECOVERED	CHLORIDE RECOVERED	CHLORIDE ABSORBED	CHLORIDE ABSORBED	CHLORIDE IN SOLUTION ABSORBED
	per cent	*cc.*	*cc.*	*cc.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*	*per cent*
1–11	0.1	50	40	10	20	50	0.16	64	−14	0	0
1–11	0.2	50	28	22	44	100	0.24	67	33	33	0.15
1–11	0.3	50	26	24	48	150	0.36	93	57	38	0.23
1–12	0.4	50	23	27	54	200	0.45	103	97	48.5	0.42
1–12	0.5	50	23	27	54	250	0.53	122	128	51.2	0.54
1–12	0.6	50	20	30	60	300	0.56	112	188	63	0.63
1–13	0.7	50	24	26	52	350	0.67	161	189	54	0.79
1–13	0.8	50	28	22	44	400	0.72	201	199	49.7	0.71
1–13	0.9	50	27	23	46	450	0.81	219	231	51.3	1.0
1–14	1.0	50	30	20	40	500	0.92	276	224	44.8	1.12
1–14	1.1	50	35	15	30	550	1.01	353	197	35.8	1.3

Above figures represent average results of four experiments carried out on two dogs.

gut approximates the percentage of chloride introduced. Goldschmidt and Dayton (1919) found this figure to be somewhat higher (0.7 per cent). Apparently at this salt concentration an equilibrium is established between the fluids on both sides of the gut wall.

From the figures showing the amounts of fluid and chloride absorbed the percentage of chloride in the absorbed fluid has been calculated. There will be noted an increased concentration of salt in the fluid absorbed as we increase the salt concentration of the fluid introduced, in spite of our having neglected the amounts of fluid and of chloride secreted. In the following experiments on the small intestine where the secretion was determined and

allowed for the correspondence between concentrations of salt in fluid introduced and fluid absorbed is even more striking.

Effects of varying the osmotic pressure of contents in the small intestine. These experiments as well as all subsequent ones were carried out on Thirty-Vella loops about two feet long. Two weeks after the operation the abdominal wound was healed in both animals, and the experiments on the small intestine then followed.

Fluids were always introduced into the proximal end of the loop and recovered from its distal end. The animals lay quietly on the table, at times even falling asleep, but occasionally became restless towards the end of the daily experiment apparently because of sensation of hunger or full bladder. No food was given on the day of the experiment but the animals had free access to water except during the experiment. Before starting an experiment in the morning, a specimen of pure intestinal secretion was collected to determine its alkalinity and chloride content. Knowing the amount of titratable alkali in pure secretion, we determined the alkali in the solutions recovered from the gut and thus were able to calculate the amount of intestinal secretion present in the fluid recovered; the actual quantity absorbed could thus be determined. Similar corrections were made for the amount of chloride secreted. Chlorides were determined by Van Slyke's (1923) method. The alkali was determined by the addition of excess N/10 sulphuric acid to the sample, heating to boiling, and back titration with N/50 sodium hydroxide, with phenolphthalein as indicator. Before each experiment the gut was washed out with the solution to be used. Great pains were taken to see that all the washing fluid was recovered; the loop was completely emptied each time by blowing air through it. The solution used was always warmed to body temperature before introducing it into the gut. The absorption time was limited to 30 minutes alternating with a 30-minute rest period before the subsequent fluid injection.

The first series of experiments on the small intestine was to study the absorption of distilled water, hypotonic, isotonic, and hypertonic sodium chloride solutions. The figures presented in table 2 are average figures of a series of experiments of the same type carried out on one of the dogs. Similar results were obtained on the other dog; the data are not published. It will be noted that when using distilled water as the fluid in the gut, there is considerable water absorbed although not as much as when some of the hypotonic salt solutions are used. Also the calculated quantity of salt secreted is less than the quantity of salt recovered from the gut. Apparently chloride has been added not only by secretion but also by diffusion.

As the concentration of sodium chloride introduced into the gut is raised from 0.1 per cent to 3.6 per cent, several changes take place. Judging from its appearance and consistency the mucus content of the recovered fluid

TABLE 2

Effect of varying the osmotic pressure of contents of the small intestine

DATE	CHLORIDE IN SOLUTION INTRODUCED	VOLUME INTRODUCED	VOLUME RECOVERED	N./10 ALKALI IN 100 CC. SECRETION	N./10 ALKALI IN 100 CC. RECOVERED FLUID	QUANTITY SECRETED	WATER ABSORBED	WATER ABSORBED	NaCl INTRODUCED	CHLORIDE IN RECOVERED FLUID	CHLORIDE RECOVERED	CHLORIDE IN SECRETION	CHLORIDE SECRETED	CHLORIDE ABSORBED	CHLORIDE ABSORBED	CHLORIDE IN SOLUTION ABSORBED
	per cent	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*	*per cent*
5–10	0	75	43	66	19.3	12.6	44.6	59.4	0	0.28	120	0.56	70	0	0	0
4–27	0.1	75	23.5	71	23.4	9.2	61.2	81	75	0.37	88.2	0.58	53	40.5	53.6	0.07
4–27	0.2	68	14	71	42.6	8.4	61.9	91.9	135	0.43	60	0.58	49	124.5	91.1	0.20
4–27	0.3	74	8	71	47	5	71	95.8	222	0.54	43.6	0.58	29	207.3	93.3	0.29
4–29	0.4	75	9.5	73	43.8	5.7	71.1	94.8	300	0.55	51.6	0.56	31.6	280	93.3	0.39
4–29	0.5	75	17	73	51.1	11.9	70	93.2	375	0.60	103	0.56	66.8	339	90.3	0.48
4–29	0.6	75	22.5	73	41.2	12.7	65.2	86.9	450	0.65	147	0.56	71.1	374	83.1	0.57
5–2	0.7	75	7.5	57	34.2	4.5	71.5	95.5	525	0.59	44.2	0.53	23.8	504.6	96.1	0.70
5–2	0.8	75	23.5	57	40.7	6.8	68.8	91	600	0.62	145.7	0.53	39	543.3	90.5	0.79
5–2	0.9	75	42.5	57	36.6	27.3	59.8	80	675	0.68	289	0.53	145	531	78.6	0.88
5–4	1.0	75	34.5	68	20.1	10.2	50.7	67.6	750	0.80	276	0.54	55	529	70.5	1.0
5–4	1.1	75	40.5	68	21.9	14.6	49.1	65.4	825	0.85	344	0.54	78	559	67.7	1.1
5–4	1.2	75	53.5	68	20	17.2	38.7	51.6	900	0.81	433	0.54	93	560	62.2	1.4
5–6	1.3	75	57.5	58	17.7	17.6	35.1	46.8	975	0.97	557	0.58	102	520	53.3	1.5
5–6	1.4	75	58	58	15	15	32	42.6	1,050	1.0	580	0.58	87	557	53	1.7
5–6	1.5	75	63	58	18.4	20	32	42.6	1,125	1.04	655	0.58	116	586	52	1.8
5–12	1.8	75	103	80	24.8	32	4	5.3	1,350	1.12	1,153	0.55	176	373	27.6	0
5–12	2.7	75	100	80	18	22.5	−2.5	−3.3	2,025	1.62	1,620	0.55	124	529	26.1	0
5–12	3.5	75	136	80	12.9	22	−39	−52	2,625	1.84	2,502	0.55	121	244	9.2	0

Each figure in the table is an average of two experiments.

is increased with the strongly hypertonic solutions. The strong salt solutions apparently are irritating to the bowel; this may be a factor in bringing about the lessened absorption. The main factor concerned is undoubtedly the high osmotic pressure of the introduced solutions.

With both dogs an equilibrium on the two sides of the intestinal wall was established at a salt concentration between 0.6 and 0.7 per cent. That is, with solutions of 0.6 per cent NaCl and lower the percentage of chloride in the recovered fluid was higher than in that introduced; at 0.7 per cent NaCl and above, the recovered fluid was lower in chloride than that introduced. The percentage of water and chloride absorbed (i.e., the ratio $\left(\frac{\text{amount of water (or chloride) absorbed}}{\text{amount of water (or chloride) introduced}}\right)$, is at a maximum with concentrations of 0.2 to 0.8 per cent chloride in introduced fluid with one dog (table 2), and with concentrations of 0.4 to 0.6 per cent chloride with the other dog (data not published). In these zones the absorption of both water and chloride is very efficient, from 90 to 95 per cent of each being absorbed in the half-hour. Why the absorption of water should be more complete in these zones of concentration than from solutions of lower concentration is not apparent. Above the zones of maximum absorption the decrease in the percentages of water and salt absorbed, with increasing concentration of salt in introduced fluid is quite consistent.

When we consider the absolute amount of salt absorbed, we see that this increases with increasing concentration of salt in the introduced fluid up to the concentration of 0.6 per cent with one dog (not published) and 0.8 per cent with the other dog (table 1). Concentrations above 0.6 per cent in the first case do not cause any significant increase in amount of salt absorbed until we reach the strongly hypertonic solutions 2.7 and 3.6 per cent. Concentrations above 0.8 per cent in table 2 do not cause any increased absorption of salt; there is in fact a marked dropping off at 1.8 and 3.6 per cent. This behavior as well as the fact that more water is absorbed from solutions of 0.4 to 0.8 per cent chloride than from weaker solutions, is hardly consistent with the view that the process of intestinal absorption is merely one of osmosis and diffusion.

It will be noted that the percentage of chloride in the absorbed fluid is the same as that in the introduced fluid up to a concentration of 1.2 per cent. With stronger hypertonic solutions this relationship breaks down, the absorption of chloride outstripping that of water, since the high osmotic pressure of the intestinal contents holds water back while the high diffusion pressure of chloride sends it in from the lumen, undoubtedly by a simple physical diffusion in addition to whatever activities the cells may present. With concentrations of 2.7 and 3.6 per cent there is no absorption of water at all, i.e., the amount of recovered fluid, even after subtracting the amount of secretion, is greater than the amount of fluid in-

Each figure in the table is an average of two experiments.

troduced. The fact that the small intestine will absorb a chloride solution of the same concentration as that introduced, up to a concentration of 1.2 per cent, might be taken as an argument for the passivity of the intestinal wall in the absorption process. However, the data discussed in the preceding paragraph, obtained in the same experiments as were the data of this paragraph, seem to point to an active participation by the intestinal wall. We would merely call attention to the pitfalls inherent in speculations based on data obtained by our present methods of investigating the problem of intestinal absorption.

Effect of K, Ca and Ringer's solution on absorption. In our next series of experiments the effects of K, Ca and Ringer's solution were studied. As will be noted in table 3, the absorption of water and of chloride from a 0.9 per cent NaCl solution containing K (42 mgm. KCl per 100 cc.) without Ca is somewhat greater than that from a pure 0.9 per cent NaCl solution. The absorption from a 0.9 per cent NaCl solution containing Ca (24 mgm. $CaCl_2$ per 100 cc.) without K is considerably greater than from a pure 0.9 per cent NaCl solution. With Ringer's solution (bicarbonate omitted) the absorption of water and chloride is practically the same as from 0.9 per cent NaCl solution. Another set of experiments was done in which an excess of K or of Ca was obtained by making up Ringer's solution containing five times the normal amount of K and Ca respectively. Here also (data not published) an excess of K was accompanied by some increase in the absorption of water and chloride, and an excess of Ca caused a considerable increase in the absorption of water and chloride, balanced Ringer's being taken as standard. The significance of the findings is not clear.

Effect of acacia on absorption. When 0.9 per cent NaCl solution containing 6 per cent acacia was introduced into the gut, there was no significant change in the absorption of water or salt, the absorption from 0.9 per cent NaCl being taken as control. The results obtained were substantiated on both animals and were quite constant (see table 4). The absorption of 0.9 per cent NaCl cannot be explained as due to the osmotic pressure of the diffusible constituents of the cell protoplasm or blood plasma, since it is isotonic with these. It might be thought that this absorption is due to the osmotic pressure of the non-diffusible constituents of the cell protoplasm or blood plasma, but in these experiments this is roughly balanced by the acacia in the introduced fluid; still no diminution in absorption occurs. Evidently the absorption process depends upon something more than osmotic forces.

The effect of atropine on absorption. From 0.1 to 0.2 mgm. of atropine sulphate per kilo body weight was injected subcutaneously in a single dose, or into the gut in a single or divided doses dissolved in the sodium chloride solution. Its effects on the pupil and pulse rate were also noted. When

TABLE 3

Dog A. Effect of K, Ca and Ringer solution on absorption

Control NaCl, 0.86 per cent

DATE	VOLUME INTRODUCED	VOLUME RECOVERED	N/10 ALKALI IN 100 CC. SECRETION	N/10 ALKALI IN 100 CC. RECOVERED FLUID	QUANTITY SECRETED	WATER ABSORBED	WATER ABSORBED	CHLORIDE IN SOLUTION INTRODUCED	CHLORIDES INTRODUCED	CHLORIDE IN RECOVERED FLUID	CHLORIDE RECOVERED	CHLORIDE IN SECRETION	CHLORIDE SECRETED	CHLORIDE ABSORBED	CHLORIDE ABSORBED	CHLORIDE IN SOLUTION ABSORBED	SALTS INTRODUCED
Dog A																	
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*	*per cent*	
3-24-27	50	58.5	94	42	26.4	17.9	35.8	0.86	430	0.69	404	0.59	156	184	42.8	1.0	Control NaCl
3-24-27	50	46.5	94	41	20.4	23.9	47.8	0.86	430	0.70	326	0.59	120	224	52	0.9	NaCl + KCl
3-24-27	50	35.5	94	42	16	30.5	61	0.86	430	0.69	245	0.59	94	279	65	0.9	NaCl + $CaCl_2$
3-26-27	50	35	80	47	21	36	72	0.86	430	0.68	238	0.53	112	304	70.7	0.84	Control NaCl
3-26-27	50	34.6	80	46	20.5	35.9	71.8	0.86	430	0.67	232	0.53	109	307	71.3	0.85	Ringer
Dog B																	
3-25-27	75	22.5	92	32	8	60.5	80.6	0.86	645	0.73	164	0.54	43	524	81.2	0.87	Control NaCl
3-25-27	75	17	92	30	5.4	63.5	84.6	0.86	645	0.71	121	0.54	30	554	85.8	0.87	NaCl + KCl
3-25-27	75	12	92	37	4.8	67.8	90.4	0.86	645	0.73	87.6	0.54	26	583	90.4	0.86	NaCl + $CaCl_2$
3-27-27	75	24	90	37	10	61	81.3	0.85	637	0.71	170	0.54	54	521	81.5	0.85	Control NaCl
3-27-27	75	24	90	33	9	60	80	0.87	652	0.72	173	0.54	54	528	80.9	0.86	Ringer solution

The above figures represent average results of six experiments carried out on each dog.

KCl used = 42 mgm. per 100 cc. NaCl solution.

$CaCl_2$ used = 24 mgm. per 100 cc. NaCl solution.

TABLE 4

Effect of acacia on absorption

VOLUME INTRODUCED	VOLUME RECOVERED	SECRETION	N/10 ALKALI IN 100 CC. SECRETION	N/10 ALKALI IN 100 CC. RECOVERED FLUID	WATER ABSORBED	WATER ABSORBED	NaCl INTRODUCED	NaCl INTRODUCED	CHLORIDE IN SOLUTION RECOVERED	CHLORIDE RECOVERED	CHLORIDE IN SECRETION	CHLORIDE SECRETED	CHLORIDE ABSORBED	CHLORIDE ABSORBED	CHLORIDE IN SOLUTION ABSORBED	SALTS INTRODUCED
cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*	*per cent*	
75	37.6	13.3	102	36	50.8	67.7	0.89	667	0.73	274	0.54	72	465	70	0.80	NaCl control
75	33	12.4	102	38	54.4	72.5	0.91	682	0.59	195	0.54	67	555	81.2	1.02	NaCl solution
75	36	11.1	102	31	50.1	66.8	0.91	682	0.59	212	0.54	60	530	77.7	1.06	plus 6 per cent
75	43	11.2	102	26	43.2	57.6	0.91	682	0.63	271	0.54	61	472	69.1	1.09	acacia
75	45	12.4	102	28	42.4	56.5	0.91	682	0.66	297	0.54	67	471	69	1.1	
Average.....						63.3								74.2	1.04	

TABLE 5

Effect of atropine on absorption

DRUG USED	SOLUTION INTRODUCED	VOLUME RECOVERED	N/10 ALKALI IN 100 CC. SECRETION	QUANTITY SECRETED	WATER ABSORBED	WATER ABSORBED	NaCl INTRODUCED	NaCl INTRODUCED	CHLORIDE IN RECOVERED FLUID	CHLORIDE RECOVERED	CHLORIDE IN SECRETION	CHLORIDE SECRETED	CHLORIDE ABSORBED	CHLORIDE ABSORBED	CHLORIDE IN SOLUTION ABSORBED	DATE
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*	*per cent*	
NaCl control	75	44	94	13.5	44.5	59.3	0.87	651	0.77	339	0.54	73	385	59.1	0.86	3-27-27
Atropine 0.1 mgm. per kilo weight subcutaneously	75	27	94	9.9	58.9	78.5	0.87	651	0.74	200	0.54	53	505	77.5	0.85	3-27-27
NaCl control	75	26	150	5.8	54.8	73	0.9	675	0.71	185	0.65	38	528	78.2	0.96	3-28-27
Atropine 0.15 mgm. per kilo weight subcutaneously	75	16	150	3.1	62.1	82.8	0.9	675	0.73	117	0.65	20	578	85.6	0.93	3-28-27
NaCl control	50	38	82	11.6	23.6	47.2	0.87	435	0.79	300	0.53	61	196	45.1	0.83	3-30-27
Atropine 0.2 mgm. per kilo weight subcutaneously	52	28.4	82	15.4	39	75	0.87	435	0.69	196	0.53	82	321	73.7	0.82	3-30-27

Each figure in the table is an average of six experiments on two dogs.

large doses were used (0.2 mgm. per kilo), particularly when given subcutaneously, the animal exhibited slight toxic effects very soon after its administration. The pulse became very rapid, the pupils dilated, the tongue was dry and the dog became restless and vomited small quantities. The results of three typical experiments are seen in table 5. It will be seen that atropine causes a considerable increase in the absorption of both water and salt. Atropine might conceivably affect intestinal absorption in three ways, first, by its effect on peristalsis, second, by its effect on the intestinal circulation, and third, by its effect on parasympathetic fibres which might be assumed to affect absorption. The action of atropine in inhibiting peristalsis should cause a diminished absorption since, according to Hamburger (1896) absorption is favored by peristaltic activity. According to Cushny (1924), "In normal animals there is thus no evidence that atropine acts on the vessels or on the nerve ends in them." We therefore have no reason to suppose that a hyperemia of the gut, which might be causing the increased absorption, took place. The third possibility, which is suggested as an hypothesis simply by the exclusion of other factors which might explain the increased absorption, is that the parasympathetic fibres to the intestine exert an inhibitory influence on the active process of absorption, and that when this inhibitory influence is removed by atropine, intestinal absorption is increased.

SUMMARY

1. The chloride percentage of the fluid recovered from a loop of intestine equals that of the fluid introduced at a percentage between 0.5 and 0.6 per cent for the colon, and between 0.6 and 0.7 per cent for the small intestine.

2. The greatest percentage absorption by the small intestine of both water and chloride, and the greatest absolute absorption of water is from solutions ranging in concentration from 0.4 to 0.6 per cent in one dog and from 0.2 to 0.8 per cent in another dog.

3. The absolute amount of salt absorbed by the small intestine increases with increasing concentrations of salt in the introduced fluid, up to the concentration 0.6 per cent with one dog and 0.8 per cent with the other dog. Concentrations above 0.6 per cent with one animal do not cause any significant increase in the amount of salt absorbed until we reach the strongly hypertonic solutions 2.7 and 3.6 per cent. Concentrations above 0.8 per cent with the other animal do not cause any increased absorption of salt; there is in fact a marked dropping off at 1.8 and 3.6 per cent.

4. The percentage of chloride in the absorbed fluid is the same as that in the fluid introduced up to a concentration of 1.2 per cent. With stronger solutions the salt leaves the lumen of the gut faster than does the water.

5. The absorption by the small intestine of both water and chloride is somewhat greater from a solution containing an excess of K ions and considerably greater from a solution containing an excess of Ca ions, than it is from 0.9 per cent NaCl or Ringer's solution.

6. The absorption by the small intestine of both water and chloride from a 0.9 per cent NaCl solution containing 6 per cent acacia is the same as that from 0.9 per cent NaCl solution.

7. The absorption by the small intestine of both water and chloride is increased when atropine is administered to the animal.

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SECONDARY EXCITATION OF THE RETINA AND THE VARIATION OF THE INTENSITY OF THE RESULTING SENSATION

FREDERICK W. ELLIS

From the author's laboratory in Newton Centre, Mass.

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It is well known that when the image of a small spot of light, observed in a dark room, falls on the temporal side of the macula, near the fovea, there are usually seen by the subject of the experiment two pale blue arcs extending from the stimulated region to the optic nerve. These arcs have been well described in recent articles by Mrs. Ladd-Franklin (1926), Amberson (1924) and Troland (1920). I have found, by calculating the position of the retinal image with the constants of Gullstrand's schematic eye, that it is necessary for this image to fall within the limits of the macula in order to excite the phenomenon. If the statements of the more recent writers, like Dimmer and Greeff (1900), regarding the size of the fovea, are true, it is easy to prove that, when the blue arcs are best defined and most intense, the stimulating image falls within the fovea. It has also been proven that the arcs are the projected images in the objective field of certain bands of nerve fibres extending from the upper and lower borders of the temporal half of the macula to the optic disc.

There seems to be no reasonable doubt that the blue arcs are due to the secondary excitation of retinal elements in, or external to the bands of nerve fibres corresponding to the arcs. The nerve fibres in these bands which are the extensions of the receptors in the macula excited by the light image supply the secondary stimulus. It is not my purpose to discuss in this paper the nature of this stimulus.

In studying the effects of variations of the intensity of the light employed as the primary stimulus it is necessary to bear in mind that the oval space enclosed by the blue arcs may be wholly, or partially, filled with a bluish haze, which we may call the blue oval. The oval is a more diffused, and a less intense, effect than the arcs, but, without doubt, it has the same cause. The oval may exist alone, when the primary stimulus is feeble, or when the exciting image is nearly a point, or is a narrow line of light. I have frequently noticed this oval while looking at a distant incandescent street lamp, in the evening, when it was possible to project the oval on a dark surface, like that of a wall in shadow. The blue arcs have claimed much greater attention in the study of secondary excitation of the retina,

as their brightness is much greater than that of the oval. The production of the arcs is favored by the close approximation of a relatively large number of primarily excited nerve fibres in certain regions of the retina. As the blue oval, with little or no indication of the arcs, may be the first subjective result obtained while graduating the intensity of the light image, its importance in connection with the subject of this paper is apparent.

The apparatus which I have employed in investigating the effect on the blue phenomenon of varying the intensity and color of the light is constructed as follows. A camera for plates, 20 cm. by 25 cm., is placed in a dimly lighted or dark room. The lenses are removed from the objective tube, leaving the shutter in place. The partition between the two compartments of the double plate-holder is removed, and an opaque plate containing an adjustable slit is fitted to one side of the holder. Slides to vary the length of the image of the slit may be inserted in the other side. The light used is furnished by an incandescent lamp for use in series in street lighting, with a single vertical filament of coiled tungsten wire. To obtain the low voltage necessary for operating the lamp, the 110 volt alternating current is transformed to one of 9.6 volts. A rheostat is inserted in the secondary circuit of the transformer in order to regulate the intensity of the light furnished by the lamp. An image of the glowing filament is focused on the slit by means of a photographic lens. When colored light is desired, a slit is placed in front of the lamp, and a spectrometer prism, on a table which may be turned about a vertical axis, is placed in front of the lens which focuses the spectrum on the camera slit. The camera is extended until the slit is 33 cm. from the front of the objective tube. As I look through the empty tube I am able to focus the slit with the reading glasses which I require for presbyopia. The slit is covered with ground glass to diffuse the light and prevent diffraction. The field upon which the arcs are projected is intensely black.

The subject of the effect of variation of color on the blue phenomenon is closely connected with that of the result of changes in the intensity of the light stimulus. As the luminosity of the spectral colors of the lamp varies greatly, it might reasonably be expected that these colors do not excite the blue phenomenon to the same extent. When we compare the effects of the lower and upper limits of the visible spectrum with that of the yellow, it is easy to prove that the supposition is correct, but, when we confine our attention to upper red, orange, yellow and green, it is not so easy to note a difference in the phenomenon that is occasioned by these colors. We find a decided difference in the effects of the most refrangible, and the least refrangible red, and the phenomenon is much lessened when the upper spectral blue is the stimulus. It is hard to detect any effect with the violet, for reasons which will be given later. I have not attempted yet to make a systematic study of the comparative effects of the more luminous

colors as they exist in the spectrum of the lamp employed, for that is unnecessary for the purpose of this research. When we produce decided variation of the intensity of any one of these colors, up to a certain limit, there is a change in the intensity of the blue arcs, and oval, which they excite.

The intensity of the blue arcs is due, to some extent, to the width of the slit, but a well-marked effect may be obtained with a narrow slit. The width of the slit in these experiments has usually been $\frac{1}{2}$ mm., but sometimes less. The light, after passing through the prism and the slit is sufficiently monochromatic for our purpose. In using the apparatus the observer fixes a point, 6 to 8 mm. from the side of the slit which corresponds with the temporal half of the eye employed in the experiment. The experimenter closes the observing eye for a few seconds, opens it again for a second or two, and then closes it again, if he wishes to repeat the observation. The blue phenomenon is transitory, and fades in about a second. Instead of opening and shutting the eye, the time shutter in the objective tube may be used to regulate the exposure to the light. To study the result of varying the intensity of the light, resistance is added to the lamp circuit until the arcs are much diminished in brightness, but are still unmistakably seen, then while the eye is held in its original position, the resistance of the rheostat is suddenly cut out, and the arcs become much brighter. This is an experiment in successive contrast, and no one who has performed it can assert that the intensity of the blue arcs is uninfluenced by that of the light which causes them, as has been done by Troland (1920) and others. After the increase in the intensity of the stimulating light reaches a certain amount it is difficult to detect an increase in the brightness of the arcs due to a further increase of that of the light. This is explained by the fact that a maximal sensation may be due to a moderate stimulus. The method employed in our experiments is not calculated to reveal fine gradations in the brightness of the arcs, and it is doubtful if any method could be devised to do that, but it is perfectly possible to distinguish at least three degrees of intensity, slight, moderate, and marked, with any color up to the beginning of the blue. This is all the evidence that is required to prove that the brightness of the arcs is not independent of that of the light which excites them.

The crystalline lens and, to a less extent, the cornea and vitreous, fluoresce when acted upon with violet and ultra-violet light. In the experiments with the apparatus just described the ultra-violet is separated from the violet by the prism, and is excluded from the eye by the screen containing the slit. When the slit is illuminated with violet light the field seems to be filled with a violet-blue light, which contrasts very little in color with that of the blue arcs. This diffused light is largely due to the fluorescence of the media of the eye, but it may be partly due to irregular

refraction of the violet light. If it were not for this diffused light we should probably be able to detect the blue phenomenon without difficulty with the violet rays. The lack of contrast between the violet-blue diffused light, and the pale blue of the arcs tends to make the arcs indistinguishable, but I have sometimes seen the blue oval when the violet light employed as the stimulus was sufficiently intense. The luminosity of the violet end of the spectrum is so slight that we could not expect, under the best of conditions, to see the blue phenomenon more than slightly marked when excited by light from this region.

Red light is ordinarily employed to excite the blue arcs, and it is especially well adapted for this purpose on account of the contrast between the blue color of the arcs and that of the slight amount of diffused red light in the eye. The brightness of the arcs caused by red light depends, to a considerable extent, on the position of the light in the spectrum. If, after we have obtained the arcs with the least refrangible red rays, we suddenly shift to the upper red, the arcs visibly brighten. The change in the light may be made by turning the prism table, or by shifting the camera laterally.

Troland (1920) published an elaborate paper in which he asserted that visual response, as evidenced by the blue arcs, follows an all-or-none law, and based his conviction on the behavior of the arcs under the varying conditions of his experiments. In the light of my own experiments, which may be repeated by anyone, I am confident that his conviction was unwarranted.

SUMMARY

An apparatus is described for studying secondary excitation of the retina when monochromatic light is used as the primary stimulus.

It has been proven with this apparatus that, when the primary stimulus is of weak or moderate intensity, the sensations excited by the secondary stimulation of the retina vary in intensity, and do not follow an all-or-none law, as has been claimed.

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THE PHYSIOLOGICAL ACTION OF CHOLESTEROL[1]

MARTIN F. CASHIN[2] AND VLADIMIR MORAVEK[3]

From McGill University Clinic, Royal Victoria Hospital, Montreal

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A vast amount of literature has accumulated on the different metabolic effects produced by changes in body cholesterol due to feedings. The results of the various observers vary greatly. However, no attempt will be made here to review the literature from this point of view.

That the animal organism is capable of synthesizing cholesterol regardless of feeding is evident from the excellent paper of Channon (1925). The intravenous injections of cholesterol have received less attention due, primarily, to the difficulty of preparing the substance in a colloidal solution. Klotz (1916) injected cholesterol held in oily vehicles and observed many interesting vascular changes, which he accredited to the cholesterol. Robertson and Burnett (1918) studied the influence of intravenous injections of cholesterol held in sodium oleate and dilute alcohol-water on tumors in laboratory animals. The only effect which they observed was that the tumors grew more rapidly. They made no note of any physiological phenomena following their injections. Stern (1926) injected cholesterol, prepared by dropping an alcoholic solution of it into boiling water, into laboratory animals and patients suffering with various diseases. He also used Gehe's colloidal solution, but does not mention the technique of its preparation. Apart from recording the cholesterol content of the blood before and after the injections, he is silent. Physiological phenomena were apparently absent, or passed unobserved, except in one case of pernicious anemia in which he noted tachycardia, dyspnea and chills following massive injections.

Moravek (1927) succeeded in preparing a colloidal solution of cholesterol which has made the study of the physiological effects of the pure substance possible. The technique for the preparation of the solution which we used is here given in detail.

Preparation of solution. The desired percentages of cholesterol and lecithin are placed in a mortar and dissolved in a small quantity of ether.

[1] The expenses of this research were defrayed in part by a grant from The Banting Research Foundation and in part by McGill University Clinic.

[2] Working under a grant from The Banting Research Foundation.

[3] Research Guest, Department of Botany, McGill University, Montreal.

The ether is then evaporated by rapid stirring. Another portion of ether is added and the process repeated. Total evaporation of the ether is assured by placing the mortar in a water bath for some minutes.

A solution of gelatin (Eastman "ash-free") in normal saline is added to the cholesterol and lecithin mixture at a temperature of 25 to 30°C. It is essential to add the solution very gradually (3 to 5 drops at a time).

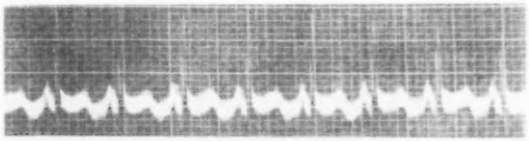

Fig. 1.

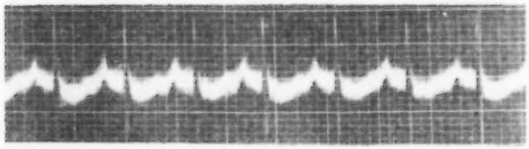

Fig. 2.

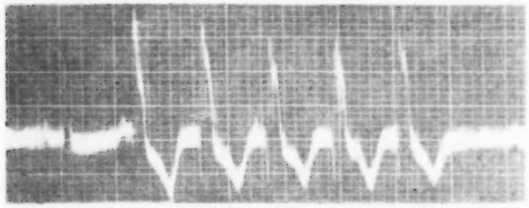

Fig. 3.

Fig. 1. Normal electrocardiograph from cat. Pulse rate 210 per minute.

Fig. 2. Electrocardiograph following 2 cc. 3 per cent cholesterol solution intravenously, showing change in form of T-wave and lengthened P-R interval. Rate 180.

Fig. 3. Electrocardiograph from same cat showing the development of short runs of ventricular tachycardia made up of right ventricular extrasystoles. The sinus rhythm maintains the same lengthened P-R as in figure 2.

Absolute homogeneity of the mixture must be procured by prolonged stirring after each addition. Particular attention should be given to ensure the thorough mixing at the point where the solid state reverses to the liquid. Having reached this point all the remaining gelatin solution may be added at once.

For the purpose of intravenous injection of the solution it is necessary to remove all the larger particles by centrifuging at 2,000 revolutions per

minute for a short time. If the mixing has been carefully carried out a minimal number of particles will have to be removed in this way.

The ratio of cholesterol and lecithin in the solution may be varied, but we used for the experiments here reported 3 per cent cholesterol and 0.3 per cent lecithin in our solution.

Observations. When we injected fairly large doses of cholesterol solution intravenously, in a cat, we were at first struck with its remarkable

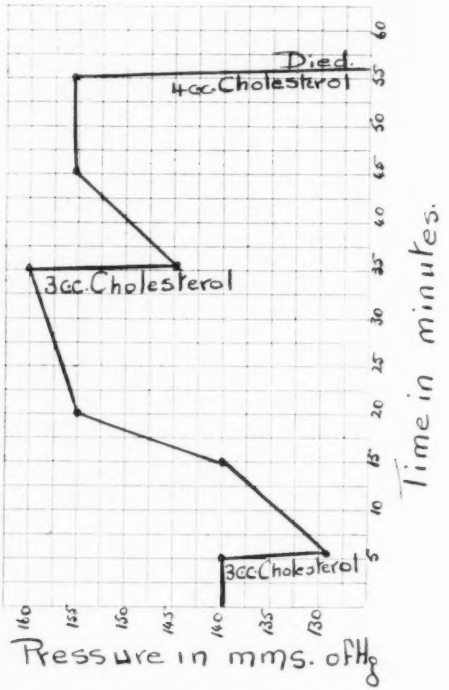

Fig. 4. Curve showing the effect of intravenous injections of cholesterol on the blood pressure.

toxicity. Six cubic centimeters of 3 per cent solution of cholesterol containing 0.3 per cent lecithin caused death in half a minute. Large doses of 0.3 per cent lecithin intravenously had no effect. We furthermore noted that the toxicity varied as to the percentage of lecithin used in preparing the cholesterol solution. In other words, the lower the content of the solution in lecithin the more toxic it was.

We found that by giving smaller doses the animal could be made to sur-

vive. The blood cholesterol was estimated by Bloor's (1916) method immediately before the injection and from 1 minute to 1 hour after. In every case the blood cholesterol dropped appreciably, the drop varying from 20 to 60 per cent. Following the injection the animal became insensitive to pain, and paralysed. So anesthetic was this action that abdominal operations could easily be performed without any reaction on the part of the animal. This anesthetic reaction was, however, a fleeting one, lasting usually 20 minutes. The anesthesia and paralysis both passed off and the cat was to all intents and purposes normal again. Immediately following the injection the respiratory rate increased enormously and the heart rate decreased. In some instances the remarkable phenomenon of the respiratory rate being double the pulse rate was observed. This ac-

TABLE 1

Cholesterol content in milligrams per cent of lungs, liver and spleen in normal cats

NUMBER OF CAT	SPLEEN	LIVER	LUNGS
	mgm. per cent	*mgm. per cent*	*mgm. per cent*
1	375	170	340
2	912	344	800
3	600	312	560

TABLE 2

Cholesterol content of lungs, liver and spleen in cats following intravenous injections of cholesterol solution

NUMBER OF CAT	SPLEEN	LIVER	LUNGS
	mgm. per cent	*mgm. per cent*	*mgm. per cent*
7	220	482	1095
8	129	416	1104
9	154	501	1111

celeration coincided with the duration of the anesthetic effect. Following the injection the blood pressure showed a preliminary drop, which was followed by a gradual and permanent rise, as indicated in figure 4. The surface tension of the blood was increased from 52 to 55 dynes following a 2 cc. injection. The fragility of the red blood cells was unchanged. It was thought that an alkalosis would be produced due to the increased respiratory rate and depth. However, in all cases so far, a moderate to very severe acidosis has been found. The effect of the injection on the heart as shown by the electrocardiograph is very interesting, and in those cases in which the animal survives the effect is also transient. These toxic actions are evidenced by marked prolongation of the P-R interval, inversion of the T wave, extra systoles occurring in bigeminae, also short par-

oxysms of ventricular tachycardia and, in some cases, complete heart block (figs. 1, 2 and 3). It was thought that both the respiratory and cardiac manifestations were due to a toxic effect through the central nervous system, or that possibly a state of anoxemia had occurred. In two animals the vagi were cut and it was found that cholesterol injection was then much less toxic. The respiratory phase deepened but did not quicken appreciably, and almost lethal doses were necessary in order to produce the cardiac phenomena. In cats with the vagi cut the lethal dose was also very much increased.

The fact that the blood cholesterol dropped following injection naturally led us to think that the pure substance had in some way mobilized the blood cholesterol, so we undertook a preliminary analysis of various organs in order to find where it had accumulated. This procedure entails so many analyses and so enormous a differentiation that obviously the field has just been broken, but as far as it goes it is interesting.

Table 1 shows the normal ratio of cholesterol content between the lung, liver and spleen.

Table 2 shows the ratio following intravenous injections.

It will be seen from these tables that the cholesterol content of the lung was enormously increased. Histological studies of such sections failed to reveal any emboli but the cholesterol can be seen in the endothelial cells lining the alveoli (the dust cells of Aschoff). Moreover, histological study shows the tremendous compensatory emphysema, and this is also very noticeable macroscopically. In some instances so marked is it that bullae appear on the lungs in an incredibly short time after the injection.

Knowing that in diabetes mellitus the cholesterol content of the blood is increased, we analysed the bloods of our experimental animals following intravenous injections of cholesterol in order to see if any disturbances had taken place in the blood sugar, but so far no appreciable change can be recorded. It had been noticed by McMeans (1916) and Klotz that vascular changes follow hypercholesteremia, but so far we are not in a position to either corroborate or refute this statement. Our animals were not injected long enough to cause any gross change.

The relation of adrenal to cholesterol metabolism has been omitted from this report and will be treated fully in a future paper.

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THE TRANSPORTATION AND ELIMINATION OF ORGANIC DYES BY THE ANIMAL ORGANISM[1]

MILDRED R. ZIEGLER AND LAFAYETTE B. MENDEL

From the Laboratory of Physiological Chemistry, Yale University, New Haven

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Many papers have been published concerning a group of dyes related to the secretory or excretory functions of a single organ; but, as far as a survey of literature indicates, few systematic studies have been made of the various possibilities of membrane permeability within an organism as a whole. In an investigation of the behavior of fat-soluble dyes in the organism, Mendel and Daniels (1912) studied the appearance of the dyes in the bile, urine and lymph. The present report involves the use of organic dyes in a study of the permeability of the membranes of the kidney, the liver, the salivary glands, the pancreas, the lymph spaces, the meninges, the ciliary body of the eye and the placenta by determining whether the injected dyestuffs traversed the cells of the gland or membrane in question, as evidenced by their appearance in the characteristic secretion thereof.

EXPERIMENTAL PROCEDURE. The dyes used in these experiments are alphabetically arranged in table 1, with their numbers as assigned by Schultz (1923) or the Society of Dyers and Colorists (1924) listed at the right. The capital letter in parentheses indicates the manufacturer.

The possible toxicity of the dyes used was first investigated, whenever any question arose in regard thereto. For this purpose rats were etherized, the skin of one leg slit and the dyestuff to be tested was injected into the femoral vein. The skin was sutured and the cut surface washed with a solution of chloramine T. The wounds healed quickly. Thus suitable dosage could be estimated. It varied from 3 to 87 mgm. per kilogram of body weight. Often the entire dose was not given at one time, but was divided into three or four smaller ones introduced at intervals during the observation. Larger doses were used in the case of dyes which are quickly eliminated from the animal. It was important to have one secretion or body fluid continually stained during each test in order to be certain that plenty of dye was circulating, thereby insuring that those glands which were not evidently eliminating the dye had the opportunity to do so.

As is well known, many dyes undergo little or no chemical change when

[1] The data in this paper are taken from a dissertation presented by Mildred R. Ziegler for the degree of Doctor of Philosophy, Yale University, 1926.

injected into the animal body, whereas others are rapidly oxidized or reduced to leuco compounds. Those of the latter class are unsatisfactory for a study of permeability unless they can be resorted to their original

TABLE 1

Alizarine VI (G)	778
Alkali blue (G)	536
Amaranth (N. A.)	168
Benzopurpurin 6 B. (G)	364
Brilliant crocein (N.A.)	227
Brilliant green (G)	499
Carmine (M)	932
Capri blue (G)	620
Crystal violet (G)	516
Dinitroresorcin (C.B.)	1
Ethyl violet (G)	518
Guinea green B (N.A.)	502
Light green yellowish, S. F. (N.A.)	505
Malachite green (N.A.)	495
Martius yellow (G)	6
Mauvein (N.A.)	688
Methyl violet (G)	515
Naphthol green B (N.A)	4
Naphthol yellow S. (N.A.)	7
Oil yellow (D)	68
Oil red (Sudan IV) (D)	232
Ponceau 3R. (N.A.)	83
Ponceau 5R. (G)	228
Pyronine G. (C.B.)	568
Spirit scarlet B. extra (Sudan II) (D)	76
Tartrazine (N. A.)	23
Thionin (G)	920*
Toluidine blue (G)	925*
Trypan blue (N.A.)	391
Yellow AB (N.A.)	22*
Yellow OB (N.A.)	61*

* Rowe (1924).
Unstarred numbers = Schultz (1923).
C.B. = Coleman-Bell.
D = Dupont.
G = Gruebler.
M = Merck.
N.A. = National Aniline Co.

state by treatment with oxidizing agents. All fluids collected in our tests were examined for leuco bases by treatment with hydrogen peroxide, if they were not colored with the characteristic shade of the dye. For those dyes with a color that might be masked by that of the bile or urine, or

mistaken for blood, the wool test was applied according to the recognized methods described by Seeker (1911), using a mordant when it was necessary.

The urines of animals which received certain fat-soluble dyes were deeply colored. It may be assumed therefore that either the animals had a lipuria or the dyes had been changed in the body so as to become water-soluble. If the color in the urine is present as the result of a lipuria, it should be easily extracted by ether, together with the fatty substance in which it would be dissolved. Ether did not extract color or fat from any of these dyed urines. Other organic solvents were utilized to remove the dye. It was found that butyl alcohol was the best solvent. The following procedure was carried out to determine whether fat-soluble dyes had conjugated in the body.

Experiment 17. The urines collected from rabbits which had been injected with Yellow OB were combined and shaken in a separatory funnel with butyl alcohol which removed the colored component. The emulsion which formed was broken by adding small amounts of ethyl alcohol. After separating and shaking several times with successive portions of butyl alcohol until all the color was extracted, the combined extracts were filtered and nearly all the alcohol distilled off. On cooling a precipitate formed in this concentrated butyl alcohol solution which was water-soluble and dyed wool yellow from an ammonia bath. Some of this precipitate treated with dilute sodium hydroxide, boiled, filtered and neutralized with hydrochloric acid gave a brownish yellow precipitate insoluble in water, soluble in ether.

By this process a water-soluble compound was extracted from the urine. When hydrolyzed it yielded a product insoluble in water but soluble in ether. These characteristics were typical of the original dye. In this way convincing evidence was obtained that the animals did not have a lipuria, but that the dye had been conjugated to aid in its removal from the body or to reduce its toxicity, or both.

All the dyes were injected intravenously, the water-soluble ones in physiological saline (37°C.), the fat-soluble ones in egg yolk emulsion, and the water- and fat-insoluble dyes in an emulsion of egg yolk or dissolved in sodium hydroxide. In the latter class the menstruum seemed to make no difference in the pathway traveled by the dye. Sometimes the experimental animal received only one dye; however, when the story had been learned concerning one compound, if it was possible to give another dye which would not be masked by the color of the preceding one, a second dye, and in some cases a third, was introduced. The behavior of nearly all the dyestuffs was noted on at least two species of animals.

Rats, rabbits, guinea pigs and dogs—about seventy animals in all—were used for this study. Urethane in doses of 0.1 gram per kilogram body weight was administered subcutaneously to all species except the dog. Dogs were given about 8 mgm. of morphine sulfate per kilogram. When

the urethane or morphine had taken effect the animal was etherized. The dyes were injected into the femoral or one of the external jugular veins in the rat, guinea pig and dog. In the rabbit the marginal vein of the ear was used. The dog was catheterized and lymph was collected from a cannula tied in the thoracic duct; biliary and pancreatic fistulae were made. In the case of the rat, guinea pig and rabbit the urinary bladder was exposed for expressing urine, and a temporary biliary fistula established.

Only healthy animals were used. Albumin was never found in the urine of the rabbits or dogs while under observation. Abundant excretion of urine was assured by giving water per os before, or physiological saline intravenously during, the experiments on rabbits and dogs. Sodium iodide, which appears in the urine of the normal animal within 10 minutes, was introduced intravenously just after the dye to learn whether the kidneys were functioning. Iodide was selected because it is colorless yet easily identified. The time of the first appearance of dye and iodide in each secretion was recorded; also its persistence therein during the period of study. About an hour before the animal was sacrificed pilocarpine hydrochloride in aqueous solution was given intravenously to promote secretion and thus increase, if possible, the permeability of the glands and mucous membranes. Garmus (1912) has shown that atropine, which inhibits secretion, causes the cells of the glands of a frog's skin to take up less dye than normally. When he gave pilocarpine after the dose of atropine the permeability was increased by exciting the cells, thereby causing more to be stained.

From 0.5 to 1 cc. of aqueous or vitreous humor was drawn into a hypodermic syringe to study the passage of dyes into the fluids of the eye. This was done, when the animal was under ether, by passing a hypodermic needle through the cornea or posterior wall of the eyeball, as occasion demanded. The drawn fluid was tested for dye and iodide. Later the eye was tapped again and the fluid tested as before.

Immediately after the animal was killed at the end of the experiment cerebrospinal fluid was obtained from rabbits and dogs by lumbar puncture, if possible. The contents and mucosa of the stomach, upper and lower intestinal tracts were examined for the presence of the dye. In pregnant animals the fetus was tested in the same manner.

Protocols of two typical experiments are reproduced here in detail. These illustrate how the data were procured and recorded for all the dyes.

Experiment 26. February 3, 1925. Dog 5. Male, 24.3 kgm.

8:20 a.m.	Morphine sulfate, 194.4 mgm., in aqueous solution injected subcutaneously
9:30	Etherized
11:00	Thoracic duct cannulated
	Lymph colorless

11:45	External jugular vein cannulated
11:50	Physiological saline, 500 cc., intravenously
12:00 m.	Animal catheterized; urine normal color
12:20 p.m.	*Carmine*, 1.2 grams, in egg yolk emulsion, and 500 cc. of physiological saline, intravenously
12:24	Sodium iodide, 500 mgm., in aqueous solution, intravenously
12:28	Lymph contained dye, no iodide
12:35	Physiological saline, 500 cc., intravenously
12:39	Urine contained dye and iodide
1:30	Physiological saline, 500 cc., intravenously
1:35	Urine contained dye and iodide
	Lymph contained dye and no iodide
2:15	Physiological saline, 500 cc., intravenously
	Nasal secretion contained no dye or iodide
	Saliva colorless, contained no leuco base, but iodide was found
2:30	Bile duct cannulated
3:00	Physiological saline, 400 cc., intravenously
3:30	Pancreatic duct cannulated
	Pancreatic juice contained no dye
	Bile contained no dye
	Urine contained iodide and dye
	Lymph contained dye and iodide
4:00	Physiological saline, 500 cc., intravenously
4:02	Physiological saline, 100 cc., containing 500 mgm. of sodium iodide, intravenously
4:30	Urine and lymph contained dye; bile and pancreatic juice did not
	Urine and bile contained iodide; pancreatic juice and lymph did not
4:56	Physiological saline, 500 cc., containing *Tartrazine*, 486 mgm., intravenously
5:00	Urine dyed with tartrazine
5:02	First appearance of tartrazine in the lymph
5:05	Bile contained tartrazine which dyed wool yellow from an acid bath
5:30	Pilocarpine hydrochloride, 24 mgm., in physiological saline, 300 cc., intravenously
5:32	Copious flow of saliva, which was colorless, contained iodide but no leuco base
	Nasal secretion contained no dye or iodide
5:40	Physiological saline, 500 cc., intravenously
5:45	Urine, bile and lymph dyed with tartrazine
	Pancreatic juice colorless
	Iodide in urine, bile and saliva, not in pancreatic juice and lymph
6:00	Experiment terminated
Autopsy:	Humors of eye colorless, contained iodide but not leuco base
	Stomach, upper and lower intestinal contents not dye-stained

Experiment 40. February 23, 1925. Rat 4. Male, 250 grams.

12:15 p.m.	Urethane, 0.25 gram, in aqueous solution, injected subcutaneously
12:50	Etherized
1:00	Bile duct cannulated
1:15	*Tartrazine*, 6 mgm., dissolved in 2 cc. physiological saline containing 50 mgm. of sodium iodide, intravenously
1:25	Tartrazine, 6 mgm., dissolved in 2 cc. physiological saline, intravenously

1:30	Bile contained iodide and dye
1:45	Urinary bladder exposed for expressing urine
	Urine contained dye
2:15	Bile contained iodide and dye
	Urine contained iodide and dye
2:36	*Light green yellowish SF*, 6 mgm., dissolved in 2 cc. physiological saline containing 25 mgm. sodium iodide, intravenously
2:43	Light green yellowish SF appeared in the bile
2:55	Urine contained light green yellowish SF, also iodide
3:30	Urine contained iodide and the green dye
	Bile contained iodide and the green dye
3:34	*Ponceau 3R*, 6 mgm., in 2 cc. saline, intravenously
3:50	Urine contained ponceau 3R
4:00	Cannula in the bile duct changed as the bile had stopped flowing
	Flow of bile started again. Bile was dyed red with ponceau 3R and contained iodide
4:30	Bile and urine dyed red and contained iodide
4:32	Pilocarpine hydrochloride, 0.25 mgm., in aqueous solution intravenously
4:35	Copious flow of saliva, which was colorless, contained iodide but no leuco base
4:50	Bile and urine dyed red and contained iod de
	Saliva colorless, contained iodide but no leuco base
	Experiment terminated
Autopsy:	Humors of the eye colorless. Mucosa and contents of stomach and upper and lower intestine not dye-stained.

PATHS OF ELIMINATION OF THE DYES. 1. *The urine.* All of the dyes administered in the experiments here reported, except malachite green, ethyl violet, spirit scarlet B extra (Sudan II), oil red (Sudan IV), alkali blue, brilliant green and mauvein were excreted in the urine. Sudan II, Sudan IV and alkali blue are fat-soluble and not water-soluble; hence they would not be expected to appear in the urine. Davis (1921), attempting to find satisfactory urinary antiseptics among the dyes, reported no renal excretion of any of the nine triphenylmethane dyes which he used. Included in his group were malachite green, brilliant green and ethyl violet, which did not show in the urine; but methyl violet, which he reported as never occurring in the urine, was found there in the present study. It seems that this disagreement of results is due to the dosage rather than to impurities or to a chemical difference in the dye,—a source of error which must be kept continually in mind. A 5 mgm. dose of methyl violet dyed the urine of the rabbit, whereas a 20 mgm. quantity resulted in anuria followed by the death of the animal in 1 hour 20 minutes. The dog tolerated this larger amount, but did not eliminate it in the urine in 3 hours. Davis stated that 20 mgm. per kilogram for the rabbit produced anuria. From the foregoing it would seem that if methyl violet could be eliminated when only small doses are given, there should be no reason why ethyl violet should not be excreted in the urine under similar conditions; however,

this is not the case. The administration of 4 mgm. per kilogram failed to color the urine. According to Davis the lethal dose of ethyl violet is 20 mgm.; he obtained no kidney excretion with 8.

Chemical radicles make a difference in the elimination of dyes. When Sudan III has two sulphonic acid groups attached to the β naphthol group of its molecule the resultant compound is brilliant crocein. This dye is water-soluble; therefore it is readily eliminated by the kidney. The possible part groups may play in the elimination of dyes will be considered later.

The malachite green seemed to be toxic to the rabbit; hence experiments with it could not be carried on for any great length of time. We used a small dose, 3 mgm. per kilogram. Dawson and Ivy (1925) have used a much larger amount in their study of the elimination of dyes by the gastric mucosa of the dog. Davis found the lethal dose for the rabbit to be 20 mgm. No statement of the appearance of malachite green in the urine has been found. Mauvein was not found in the urine in any experiments, although it is slightly soluble in water and more so in the presence of acid.

Considering those dyes which were eliminated in the urine, one finds that three,—yellow AB, yellow OB and oil yellow,—were fat-soluble. The urine collected from animals which had received these fat-soluble dyes was examined according to the method already described. It was found that the dyes had conjugated with some group or compound which had converted a water-insoluble substance into a dye soluble in water. Salant and Bengis (1916), performing experiments with oil-soluble dyes, found that they were eliminated in the urine. This was contrary to work previously reported from this laboratory (Mendel and Daniels, 1912). These investigators never found fat-soluble dyes in the urine unless an alimentary lipuria arose. Further investigation by Read confirmed this observation.

Three dyes, namely, alizarine VI, dinitroresorcin and carmine, were water- and fat-soluble. The two former were given dissolved in dilute alkali; hence they were possibly present in the urine as their soluble salts. The latter, acid in character, probably formed a soluble salt with some of the reserve alkali of the blood.

The other eighteen dyes were readily water-soluble; therefore they would be expected to show in the urine. The appearance of the colors in the urine was observed within 5 to 94 minutes after they were injected intravenously in the rabbit and within 3 to 19 minutes in the dog. Elimination usually continued during the period of the experiment, which varied from 32 minutes to $7\frac{1}{2}$ hours; however, observations on some animals showed that elimination may continue for days. After a dose of 50 mgm. per kilogram in a rabbit, trypan blue continued to be excreted in the urine for 11 days. The dose has a marked effect on the duration of elimination.

2. *The bile.* Many dyes have been studied in connection with problems of liver function. The ideal substance for this purpose would be one that is

eliminated through the liver only. References to dyes eliminated in the bile will be found in studies by Abel and Rowntree (1909–1910), Mendel and Daniels (1912), Salant and Bengis (1916), Delprat (1923), Tada (1923), Tada and Nakashima (1924), Rosenthal (1922), and Rosenthal and White (1924).

In the present study dinitroresorcin and carmine were the only dyes not secreted into the bile, but they appeared in the urine and lymph. Both are insoluble in water and fat; they were given dissolved in dilute alkali and egg yolk emulsion. The dyes are unlike in chemical structure. All the other dyes investigated appeared in the bile. From 2 to 90 minutes after injection into the blood stream of the rabbit, and from 6 to 145 minutes in the case of the dog, the dyes were found in the bile. The dose of dye given was sufficient in most cases for its elimination to continue throughout the duration of the experiment.

3. *The pancreatic juice.* Apparently little attention has been paid to the rôle the pancreas may have in dye elimination. Using a dog with a permanent pancreatic fistula Giichiro (1923) tested the pancreatic juice for the presence of indigo-carmine, neutral red, methylene blue, tetrachlorphthalein, acridine red, congo red, rhodamin, eosin and trypan blue respectively after their subcutaneous administration. A full meal was given the animal during the experiment to assure a copious pancreatic secretion. Methylene blue was the only dye which was found in the pancreatic juice. Secretion began in 40 minutes and persisted for 150 minutes.

In our study alizarine VI, alkali blue, amaranth, brilliant crocein, carmine, crystal violet, dinitroresorcin, ethyl violet, Guinea green B, Martius yellow, methyl violet, naphthol yellow S, ponceau 3R, pyronine G, spirit scarlet B extra, and tartrazine were injected intravenously into dogs with temporary pancreatic fistulae. Amaranth, Martius yellow, and methyl violet, dyes of unlike chemical structure but all water-soluble, appeared in the pancreatic juice. Secretion began in 59, 130 and 145 minutes respectively. Pilocarpin given toward the end of each experiment served to increase the activity of the pancreas if the juice was flowing slowly.

It is of interest here that the same factor which prohibited the excretion of ethyl violet in the urine of rabbits, and allowed the passage therein of methyl violet, apparently controls the non-secretion of ethyl violet and the secretion of methyl violet respectively in the pancreatic juice of the dog

4. *The saliva, tears and nasal secretion.* All of the dyes selected for our study were tested for their reappearance in the secretion of the salivary glands, but none was ever found there either unchanged or as a leuco compound. The elimination of these dyes by the saliva evidently does not occur. Why the salivary glands are such an efficient barrier to dye secretion remains undetermined. Perhaps the size of the molecule is important,

as all the known constituents of saliva except mucin are of low molecular weight and of comparatively simple structure. No systematic study of the elimination of dyes in connection with salivary secretion has been found. Sollmann (1924) reported that phenolphthalein occurs in saliva and tears after intravenous injection. We have confirmed this with 0.06 cc. of a saturated alcoholic solution of phenolphthalein injected intravenously into a rabbit. It was found in the saliva. When this result was compared with Sollmann's report, it served as a check, indicating that our technique was not at fault. Injected iodide was always present in the saliva in these experiments even when given in as small a dose as 0.01 mgm. per kilogram to the rabbit. It was also found in the tears and nasal secretion. The dyes were never present in the saliva.

Pilocarpin hydrochloride in aqueous solution injected intravenously produced the expected copious flow of saliva, tears and nasal secretion. Since this did not facilitate the salivary elimination of the dyes, it was thought that possibly direct electrical stimulation of the chorda tympani might cause them to appear in the saliva. Accordingly a dog was given morphine, etherized, and an external jugular vein and the salivary ducts were cannulated. Picric acid, which was selected because of its small molecular weight, light green yellowish SF, brilliant crocein, and alkali blue were injected into this dog. Direct electrical stimulation of the chorda tympani failed to produce elimination of the above mentioned dyes in the saliva.

5. *The gastric and intestinal mucosa secretions.* At autopsy the mucosa and contents of the upper and lower intestinal tracts were always examined for evidences of gastro-intestinal elimination of the dyes. The tracts were deeply stained when the animals had received naphthol yellow S, crystal violet, methyl violet and ethyl violet. Evidence obtained by this method is not very satisfactory; it can only serve as a guide to more accurate work which should be conducted on animals with Pawlow pouches and Thiry-Vella intestinal loops. Investigations on animals with Pawlow pouches have been made by Saxl and Scherf (1922), Finkelstein (1923) and Dawson and Ivy (1925). It was impossible to determine definitely from our autopsies whether the dye entered the stomach, thence passing into the intestines, or whether it was perhaps regurgitated from the intestine into the stomach. Since the mucosa was stained in both the stomach and the intestine in each case, it would seem plausible that the dye was eliminated by the glands in the mucosa. Thionin and toluidin blue, which Dawson and Ivy found were eliminated by the gastric mucosa of the dog, did not appear to be secreted by the mucosa of the rabbit. The difference is probably due to the much smaller dose given in the present study rather than to the different species of animal.

DO THE DYES PASS INTO OTHER BODY FLUIDS AND SECRETIONS? 1. *The*

cerebrospinal fluid. The permeability of the meninges and choroid plexus as well as the mechanism of formation of cerebrospinal fluid can be studied by determining what foreign substances can pass into the fluid. According to Jervell (1925), in cases of meningitis uranin entered the fluid within 3 hours after intramuscular injection or per os administration. Basing his conclusions on seventy-six cases, he states that the cerebrospinal fluid is an exudate, not a secretion, inasmuch as uranin passes readily into exudates. Achard and Ribot (1909) identified iodide in spinal fluid 5 to 15 hours after administration. Catton (1916) never found it following daily large doses (6 grams) continued for 11 weeks, or a single dose of 17 grams. Hald (1911) states it is present, though not constantly.

In our study cerebrospinal fluid from dogs and rabbits was obtained free from blood after the administration of iodide and one of the following dyes: alizarine VI, amaranth, carmine, oil red, pyronine G, tartrazine, trypan blue, ponceau 3R, phenolphthalein, or yellow AB. In no instance did iodide or dye in any form appear in the cerebrospinal fluid.

2. *The lymph and tissues.* Dyes have been used to determine whether the lymphatics are developed from tissue spaces or whether according to the newer conception they represent a closed system (Macleod, 1922). Mendel and Daniels (1912) studied the transport of fat-soluble dyes by the lymph. They found that these dyes dissolved in fat enter the body through the lymphatics.

In this study lymph was collected after the intravenous injection of the following dyes:

Alizarine VI
Alkali blue
Amaranth
Brilliant crocein
Carmine
*Crystal violet
Dinitroresorcin
*Ethyl violet
Guinea green B
Light green yellowish SF
Martius yellow
*Methyl violet
Naphthol yellow S
Ponceau 3R
Pyronine G
Spirit scarlet B extra
Tartrazine
Yellow AB
Yellow OB

All except those starred appeared therein within from 2 to 25 minutes after injection. Guinea green B, light green yellowish SF, tartrazine, yellow AB, and yellow OB were excreted in the urine first; the others appeared in the lymph before they were found in the urine.

Amaranth, methyl violet, ethyl violet and light green yellowish SF stained the skin, mucous membranes and muscles. Evidently methyl violet and ethyl violet diffused from the blood directly to the tissues; it was impossible to identify them in the lymph. Yellow AB, yellow OB, oil yellow, oil red and spirit scarlet B stained the body fat by virtue of their solubility in fat. Their presence in other tissues was not identified.

3. *The fetus and amniotic fluid.* The mechanism by which food and waste products are passed from the maternal blood through the placenta to the fetal blood is not perfectly understood. Dyes have been used extensively in studying the permeability of the placenta. Hofbauer (1905) assumed that dye, which he found in the blood of the fetus, had separated from fat metabolized by the embryo. Gage and Gage (1908) and Mendel and Daniels (1912) could not demonstrate that fat-soluble dyes entered the fetus from the mother. Shimidzu (1922), investigating the permeability of the placenta to twenty-three water-soluble dyes, showed that all the basic dyes examined and eight acid dyes penetrated the placenta, while eight other acid dyes could not be found in the fetus. He states that the power of dyes to pass the placenta is related to their colloidal state. Wislocki (1921), studying the permeability in the reverse direction (from the fetus to the mother), learned that trypan blue, a colloidal dye, injected into the fetus never appeared in the maternal urine, while phenolsulphonephthalein, a crystalloid, appeared in the maternal urine after fetal injection.

In this phase of our study the thirteen dyes listed below were given to pregnant rabbits:

Alizarine VI	Phenolphthalein
Carmine	Ponceau 3R
Capri blue	Pyronine G
Dinitroresorcin	Tartrazine
Malachite green	Thionin
Oil red	Trypan blue
Oil yellow	

After periods of ½ to 3½ hours had elapsed the animals were killed. The amniotic fluid and fetus were examined for the dye and injected iodide. It was not possible to identify the dye in either the amniotic fluid or the fetus. When iodide was found in the amniotic fluid it was in the fluid which surrounded the most mature fetuses. Those whose crown-rump measurement was 11 to 27 mm. gave no test for iodide in the amniotic fluid, while those which measured 72 to 91 mm. showed iodide. Shimidzu found that the placenta was slightly permeable to alizarine, but his experiment lasted 12 hours, a considerably longer time than ours. Inasmuch as the iodide penetrated the placenta in a very short time, one would expect that the dyes would pass similarly.

4. *Humors of the eye.* Lewis (1916) studied the passage of trypan red and trypan blue into the humors of the eye of the rabbit after intravenous injection. After several minutes the anterior chamber fluid was slightly colored; it reformed very rapidly, and was then much more intensely stained.

Table 2 gives a list of dyes tested for their appearance in the fluid of the anterior chamber of the eye in this research. Crystal violet and toluidin blue did not appear in the humor in the second tapping although the fluid had reformed. Ethyl violet and naphthol green B stained the anterior surface of the iris before the first fluid was drawn.

THE EFFECT OF INCREASING THE PERMEABILITY OF THE CAPILLARIES. Four experiments were carried on to determine whether by altering the permeability of the capillaries by a known "capillary poison," the dyes would take unusual pathways of excretion. The plan of each experiment was the same as outlined under "Experimental Procedure" except that "peptone" or histamine was given immediately after the dye. Alkali blue was given to rabbits in histamine and peptone shock, and to a dog in peptone shock. Ethyl violet was given to a rabbit in histamine shock. Blood pressure tracings recorded the degree of shock in the dog. Both of these

TABLE 2

Dyes tested for appearance in fluid of the anterior chamber of the eye

NAME	FIRST TAPPING	MINUTES BETWEEN TAPPING	SECOND TAPPING	ANIMAL
Ethyl violet	Colorless	50	Colored	Rabbit
Naphthol green B	Colorless	6	Colored	Rabbit
Crystal violet	Colorless	45	Colorless	Dog
Martius yellow	Colorless	5	Colored	Dog
Brilliant crocein	Colorless	30	Colored	Rabbit
	Colorless	45	Colored	Dog
Alkali blue	Colorless	15	Colored	Rabbit
		140	Colored	Rabbit
Toluidin blue	Colorless	40	Colorless	Rabbit

dyes normally appear in the bile but not the urine. The "capillary poisons," histamine and peptone, did not cause alkali blue or ethyl violet to appear in the urine, in the saliva, the aqueous humor after tapping, the contents of the stomach or upper and lower intestinal tracts, or to stain the mucosa of the alimentary tract. Alkali blue was not excreted in the pancreatic juice of the dog. Although the capillaries are known to be affected in peptone shock (Underhill and Epstein, 1923), and show a changed permeability, it did not cause any alteration in the excretion or secretion of the dyes by the various membranes and glands in these experiments. Siengalewicz and Clark (1924) could not induce the passage of trypan blue into the brain substance by production of peptone shock, although the passage of the dye from the blood stream into the peritoneal cavity proceeded at a variable rate.

WHAT FACTOR DETERMINES THE PATH OF ELIMINATION FOR THE DYES? In order to explain why some dyes permeated one membrane and not

another, the dyes used have been grouped according to different physicochemical properties and considered in connection with their elimination and course travelled in the body. The dyes, classified in respect to solubility, include five that are fat-soluble, four which are water- and fat-insoluble, and twenty-two water-soluble. Not all the dyes in any one class behaved in the same manner. This is also true if they are grouped according to their chromophores. There are eight triphenylmethane-, two thiazine-, two nitroso-, twelve azo-, and two nitro-dyes. Those with other chromophores are represented by only one dye each. The acid dyes, with the exception of carmine, oil red, and spirit scarlet B, were all excreted in bile, urine, and lymph. Of the basic dyes, six were found in urine and bile,

TABLE 3

Dyes grouped according to solubility

Fat soluble dyes	*Water insoluble dyes*
Oil yellow	Alizarine VI
Sudan II	Carmine
Sudan IV	Dinitroresorcin
Yellow AB	Mauvein
Yellow OB	
Water soluble dyes	
Alkali blue (also fat soluble)	Martius yellow
Amaranth	Methyl violet
Benzopurpurin 6B	Naphthol green B
Brilliant crocein	Naphthol yellow S
Brilliant green	Ponceau 3R
Capri blue	Ponceau 5R
Crystal violet	Pyronine G
Ethyl violet	Tartrazine
Guinea green B	Thionin
Light green yellowish SF	Toluidin blue
Malachite green	Trypan blue

and four in the bile only. In the group of fat-soluble dyes the acid ones appeared only in the bile and the basic in the bile and urine. The presence of an $-NH_2$ group caused the basicity of the basic fat-soluble dye. Possibly it is this group which is capable of conjugation, thereby rendering the fat-soluble dye water-soluble. The fat-soluble dyes, yellow AB and yellow OB, become conjugated, but not with sulfuric acid.

Schmiedeberg (1878) showed that aniline given to animals is eliminated partly as the glucuronate and partly as the sulfate. Gautrelet and Gravellat (1907) claim that marine blue unites with sulfuric acid. According to Salant and Bengis (1916) benzeneazophenol and Sudan G, both fat-soluble dyes, are excreted in combination with glucuronic acid.

All dyes of this study which are sodium sulphonate salts are eliminated

in the bile, urine and lymph. Alkali blue, which has a sulphonic acid group as such and is not a salt, is excreted by the bile only. The addition of a sodium sulphonic acid group to a fat-soluble dye, which was eliminated only in the bile and lymph, made it water-soluble and caused it to be excreted in the urine. Sudan III by the addition of two sodium sulphonic acid groups becomes brilliant crocein. If three sulphonic acid groups are added to Sudan III we have ponceau 5R. Both of these dyes are water-soluble; both are excreted in the bile and urine. It is not the solubility of dyes alone which changes the pathway of excretion, for the water-soluble dyes, alkali blue, ethyl violet, and brilliant green, which do not have a

TABLE 4

Dyes grouped according to chromophores

1. Azo dyes
   - Amaranth
   - Benzopurpurin 6B
   - Brilliant crocein
   - Oil red
   - Oil yellow
   - Ponceau 3R
   - Ponceau 5R
   - Spirit scarlet B extra
   - Tartrazine
   - Trypan blue
   - Yellow AB
   - Yellow OB
2. Triphenylmethane dyes
   - Alkaline blue
   - Brilliant green
   - Crystal violet
   - Ethyl violet
   - Guinea green B
   - Light green yellowish SF
   - Malachite green
   - Methyl violet
3. Thiazine
   - Thionin
   - Toluidin blue
4. Nitroso
   - Dinitroresorcin
   - Naphthol, green B
5. Nitro
   - Martius yellow
   - Naphthol yellow S
6. Natural—carmine
7. Anthracene—alizarin
8. Azine—mauvein
9. Oxazine—capri blue
10. Xanthene—pyronine G

sulphonic acid group, are not eliminated by the urine. Apparently the sulphonate group makes urinary excretion possible by furnishing a means of conjugation with other radicles.

Six of the dyes which are chlorides were present in both bile and urine, three in bile only.

Consideration of the dyes containing -OH groups showed that all fat-soluble ones with an -OH group are excreted by the bile only, the water-soluble by the bile and urine, the one water-insoluble in the urine only, and the other in the bile and urine. All dyes containing an -OH radicle were found in the lymph when it was examined. Dyes with $-NH_2$ groups not

substituted and those containing both $-NH_2$ and -OH groups in the molecule were eliminated in the urine, bile and lymph. Alkali blue which contained substituted and unsubstituted amino groups was found only in the urine. Ten of the dyes used contained substituted amino groups. Four appeared in the bile only, the others in the urine and bile, but not always in the lymph.

The presence of a dye in the different body fluids might depend upon the fact that some membranes or glands are permeable to the free dye and others to its salt. Experiments were, therefore, carried on in vitro with the dyes characterized with irregularities of behavior: Yellow AB, yellow OB, mauvein, malachite green, brilliant green, crystal violet, methyl violet, oil yellow, oil red, Sudan III, spirit scarlet B and alkali blue. They

TABLE 5

Dyes grouped according to their acidic and basic properties

Acidic dyes	*Basic dyes*
Alizarine VI	Alkali blue
Amaranth	Brilliant green
Benzopurpurin 6B	Capri blue
Brilliant crocein	Crystal violet
Carmine	Dinitroresorcin
Guinea green B	Ethyl violet
Light green yellowish SF	Malachite green
Martius yellow	Mauvein
Naphthol green B	Methyl violet
Naphthol yellow S	Oil yellow
Oil red	Pyronine G
Ponceau 3R	Thionin
Ponceau 5R	Toluidin blue
Spirit scarlet B	Yellow AB
Tartrazine	Yellow OB
Trypan blue	

were used to determine whether the presence of the dye in water or fat solution could be changed by the addition of acid or base to the mixture. A few milligrams of dye were placed in a test tube containing about 5 cc. of water and 5 of fat (olive oil or cod liver oil). To the mixture was added 2 cc. of tenth normal acid. The whole was well shaken and allowed to stand. After several days 2 cc. of tenth normal alkali were added, the mixture shaken and allowed to settle. If the dye was originally soluble in fat it remained so in the presence of both acid and base. Oil red, oil yellow, yellow OB, and yellow AB showed an exceedingly slight soluble fraction in the water of the basic mixture, but by far the greatest amount of the dye was in the fat. The water-soluble fraction was negligible. It could hardly account for the large amount of excretion of the three

TABLE 6

Elimination of dyes according to the chemical groups they contain

DYE	BILE	URINE	LYMPH
A. Sulphonic acid			
Alkali blue	+	−	+
Amaranth	+	+	+
Benzopurpurin	+	+	
Brilliant crocein	+	+	+
Guinea green B	+	+	+
Light green SF	+	+	+
Naphthol yellow S	+	+	+
Naphthol green B	+	+	
Ponceau 3R	+	+	+
Ponceau 5R	+	+	
Tartrazine	+	+	+
Trypan blue	+	+	
B. Chloride			
Capri blue	+	+	
Crystal violet	+	+	−
Ethyl violet	+	−	−
Malachite green	+	−	
Mauvein	+	+	
Methyl violet	+	−	
Pyronine G	+	+	+
Thionin	+	+	
Toludin blue	+	+	
C. -NH_2 not substituted			
Benzopurpurin 6B	+	+	
Oil yellow	+	+	
Thionin	+	+	
Toluidin blue	+	+	
Trypan blue	+	+	
Yellow AB	+	+	+
Yellow OB	+	+	+
D. Substituted NH_2			
Alkali blue	+	−	+
Brilliant green	+	−	
Capri blue	+	+	
Crystal violet	+	+	−
Ethyl violet	+	−	−
Guinea green B	+	+	+
Light green SF	+	+	+
Malachite green	+	−	
Mauvein	+	−	
Methyl violet	+	+	−
Pyronine G	+	+	+

TABLE 6—*Concluded*

DYE	BILE	URINE	LYMPH
E. NH_2 and -OH			
Guinea green B	+	+	+
Light green SF	+	+	+
Trypan blue	+	+	
F. OH			
Alizarine V1	+	+	+
Amaranth	+	+	+
Brilliant croceine	+	+	+
Carmine	+	+	+
Martius yellow	+	+	+
Naphthol yellow S	+	+	+
Ponceau 3R	+	+	+
Ponceau 5R	+	+	
Sudan II	+	−	+
Sudan III	+	−	+
Sudan IV	+	−	
Tartrazine	+	+	+

latter dyes in the urine, as oil red which acts the same did not appear therein. The water-soluble dyes remained in the water only. Methyl violet was an exception. It was slightly soluble in fat in the presence of the base. Alkali blue, which is only slightly soluble in cold water and fat, was dissolved in the fat in the presence of acid or base. From these experiments in vitro the question of the pathway of elimination of a dye in the body must involve its ability to conjugate with another substance rather than the existence of the dye free or as a salt. At least the means of excretion cannot be explained by any chemical or physical property here discussed.

Selective secretion or excretion takes place, but the fundamental cause is not understood. It must be an inherent factor in the cells of the membrane which results in the different permeability of the body organs. Functional activity of cells, which is known to be accompanied by changes of permeability, has been considered in these experiments. The salivary glands were activated by pilocarpin and electrical stimulation of the chorda tympani; the kidney, by giving saline intravenously, and the other glands examined, by pilocarpin. Nevertheless, the dyes could not be forced by these means into some of the secretions and excretions. It cannot be estimated to what extent functional activity influenced the elimination of the injected dyes.

CONCLUSIONS

Thirty-one dyes injected intravenously into rats, dogs and rabbits were studied to determine what body membranes they can permeate. Twenty-four of the dyes were eliminated in the *urine*. Three fat-soluble dyes,—yellow AB, yellow OB and oil yellow,—conjugated, thereby becoming water-soluble.

Dinitroresorcin and carmine, fat- and water-insoluble dyes, were the only ones not found in the *bile*.

Amaranth, Martius yellow and methyl violet, water-soluble dyes of unlike chemical structure, appeared in the *pancreatic juice*.

None of the dyes was ever found in the *saliva* either after stimulation of the salivary glands by pilocarpin or after electrical stimulation of the chorda tympani. The salivary glands were actually functioning, and injected iodide always appeared in the saliva.

Naphthol yellow S, crystal violet, methyl violet, and ethyl violet stained the mucosa of the *stomach* and upper and lower *intestinal tracts*.

Cerebrospinal fluid (when collected) was always free from the dyes or leuco bases.

Nineteen dyes were tested for their presence in the *thoracic lymph*. All except three appeared therein.

Dyes were not found in the *humors of the eye* unless the fluid had been previously withdrawn and allowed to re-accumulate. Humor reformed rapidly. Five of seven dyes deeply stained the newly formed fluid.

"Capillary poisons," histamine and peptone, did not change the normal path of excretion of alkali blue and ethyl violet.

The body membranes studied showed different degrees of permeability. No constant chemical or physical property was found which explained this difference. It is of interest that the basic fat-soluble dyes were eliminated in the urine and bile, while the acid ones were present in the bile only. It is suggested that the ability to conjugate a dye with another group in the body may be an important factor in its manner of elimination. Functional activity of the salivary glands was produced by electrical stimulation of the chorda tympani and by the administration of pilocarpin. Dyes were not secreted even when the salivary glands were active. It was not determined whether functional activity plays a part in elimination of dyes by other glands and membranes.

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THE EFFECT OF DIFFERENT AMOUNTS OF SEXUAL INDULGENCE IN THE ALBINO RAT

I. GROWTH[1]

JAMES ROLLIN SLONAKER

From the Department of Physiology, Stanford University, California

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The object of this experiment was to determine the effect of various amounts of sexual indulgence on growth, reproduction and the young, food consumption, daily and total voluntary activity and the span of life of the albino rat. The results will be considered under the above captions. The experiment was started in February 1924 and lasted until the last of the animals died a natural death in 1927.

In reviewing the literature on this subject I have failed to find any investigation which has been carried on during the entire life span of an animal. A vast amount of work has been done by different experiment stations and livestock raisers in breeding animals. These investigations, however, have been largely concerned in increasing production by developing different strains of animals or by the feeding of certain foods. They have not taken into account the effect on the span of life nor the volitional activity of the animals.

Five degrees of sexual indulgence were arbitrarily selected. These ranged from absolute abstention to a complete satisfaction of sexual desires. Group A consisted of strict abstainers in which no sexual intercourse was permitted during their entire life span. This group was composed of 6 males and 29 females. Group B was classed as light breeders. This group of 4 males and 9 females was allowed to mate and rear but two or three litters. Group C, composed of 4 males and 9 females, included medium breeders. In this group normal conditions similar to that of the wild rat were maintained as nearly as possible. That is, the mothers were allowed to nurse their young to the weaning age (twenty days) after which rebreeding occurred as soon and as often as possible during the entire breeding age. The females of this group spent the greater part of their sexual lives in gestation and lactation. Group D, composed of 4

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males and 9 females, were heavy breeders. In this group the young were destroyed after the weights were taken at birth and the mothers remated as soon as possible, which often took place the day of delivery. During the greater part of the sexual life the females of this group were in a condition of gestation. Group E consisted of animals in which excessive coitus took place without reproduction. To accomplish this a number of females were hysterectomized and placed with males of the same age. Twelve males and forty hysterectomized females were placed in four stationary cages—three males and ten females to each cage. Since hysterectomy does not interfere with the normal oestrual rhythm an average of two or more of the females were in heat each day, thus presenting many opportunities for coitus. These opportunities the males took advantage of. These males thus had coitus until completely satisfied and the females many times each fourth day during their life span. Twelve hysterectomized virgin rats and the six bachelor males were used as controls for this group. Since the operation of hysterectomy did not interfere in any way with normal growth, activity or length of life, the results of these twelve hysterectomized rats were grouped with the other virgin rats in group A.

The experiment was carried on in a large attic room containing approximately 160 square meters of floor space. It was well lighted by a skylight 25 square meters in area. A ventilating fan, which blew the air outside through the roof, caused a good change of air without drafts in any of the lower portions of the room. A recording thermometer registered the temperature at all times. This showed a fluctuation of temperature for the years of 12 degrees centigrade, ranging from a minimum of 14 degrees in the winter to a maximum of 26 degrees in the summer. The daily range was seldom greater than 5 degrees, being coldest at night when the rats were normally active and warmest during the day when they were asleep. A recording hygrometer showed a range of moisture from 40 to 70 per cent saturation, with an average of approximately 50 per cent for the winter months and 60 per cent for the summer months. The seasonal difference may be attributed to artificial heat in winter. The daily range was seldom more than 20 per cent, being higher during the night. The animals were thus kept in a room which showed no marked changes in temperature and moisture. All would presumably be affected in a similar manner by whatever changes occurred. Such changes as did occur were insufficient to produce any noticeable effect. The only variable factor was the amount of sexual indulgence which was permitted in the different groups. Any differences in the results may, therefore, be considered as due to this one variable factor.

Owing to the lack of a sufficient number of revolving cages only a limited number of animals could be tested for their volitional activity. Five females of each of the groups A, B, C and D were kept in the revolving

cages, one animal to a cage, during the entire experiment. Careful records of their daily activity, food consumption and body weight were taken and plotted in curves for each rat. Mating trials were made in the evening. Stock males were used in mating the females in the revolving cages.

Each of the females in groups B, C and D, kept in stationary cages, was always bred to the same male. That is, these females were paired at the beginning of the experiment and this arrangement maintained. In all cases the males were removed from their respective cages a day or so before delivery was expected. In group B the males were with the females only a sufficient number of times to produce two or three litters. In group C they were returned to the females about the tenth day of lactation. In group D they were again placed with the females the day of delivery of young. In group E they were with the females constantly.

The animals were fed throughout the experiment on the following carefully prepared synthetic diet: ground whole wheat, 3375; whole milk powder, 500; commercial casein, 750; sodium chloride, 50; calcium carbonate, 75; ground alfalfa, 150; butter fat, 250. These ingredients were thoroughly mixed by running through a grinder and stirring. This food apparently contained all the essential food factors as indicated by the normal growth, reproduction, and duration of life which resulted. It contained approximately 21 per cent protein, 9 per cent fat, 55 per cent carbohydrates, and 15 per cent water, salts and ash. Its fuel value was 3.94 calories per gram. The animals were fed and watered in the evening an amount greater than they would consume during the following twenty-four hours. An accurate record was kept of the individual daily food consumption of all the females in the revolving cages and plotted. This could not be easily done with the paired animals in the stationary cages. At the feeding time the number of revolutions turned by each rat was recorded and the counter set back to zero, such mating tests as desired made, and body weights taken and recorded in tabular and graphic forms. A glance at our daily activity curve enabled us to anticipate the mating time owing to the fact that oestrus is accompanied by a marked increase in spontaneous activity (Wang, 1923; Slonaker, 1924).

The 126 animals used were from the same stock of pure bred albino rats and as nearly as possible the same age. The average age at the beginning of the experiment was 74.5 days. The range in age was from 58 to 88 days. The females averaged 73.6 days of age and the males 79 days. The experiment was thus started when but few of the animals were older than the average age of sexual maturity. Most of them were still sexually immature as evidenced by the time of delivery of the first litter.

Growth. Females, group A. This group of virgin rats consisting of twenty-nine animals, was arranged in cages as follows: from the beginning of the experiment five were confined throughout their entire life span in

separate revolving cages (Slonaker, 1908); the remainder were kept in stationary cages (Slonaker, 1918). Twelve of those in stationary cages had been hysterectomized at the age of twenty days, but behaved in approximately the same manner as the other virgin unoperated rats. This group thus consisted of three divisions: 1, normal virgins in revolving cages; 2, normal virgins in stationary cages; and 3, hysterectomized virgins in stationary cages. The average weight of each of these subgroups at 100 days was 138, 156 and 134 grams respectively. They all grew in a normal manner, as indicated in figure 1, and closely corresponded until approximately 400 days of age. After this age both groups in stationary cages increased in weight more rapidly than that in the revolving cages. The gradual increase in weight continued until about 600 days after which they all showed a more rapid increase and reached a maximum at approximately 820 days. The normal virgins in stationary cages were the heaviest reaching a maximum weight of 264 grams. The hysterectomized virgins were second with a maximum of 262 grams. Those in the revolving cages had an average maximum of 242 grams. The cause for this more rapid increase in weight seems to be closely associated with the cessation of the oestrual cycles and agrees with results usually found in other animals, including the human. After this maximum there was the usual decline due to senility. An explanation of the cause for the heavier weight in the stationary cage rats seemed at first quite simple. That is, the energy which was manifested in growth in one case was partly expended in activity by those in the revolving cages. This assumption, however, was not supported by groups B, C and D, in which each of the revolving cage groups surpassed the weight of those in the stationary cages. While this explanation may be in part true some other cause is also evidently involved. It could not have been a question of food for they were all fed the same and amounts greater than they would consume between feeding times.

Females, group B. This group consisted of light breeders. The number of litters each was permitted to bear was never less than two nor more than three. The average number of litters for each was 2.64. This represents also the average sexual activity. The average rate of growth in this group (see fig. 2) was quite different from that of the virgins. The animals increased in weight much more rapidly and reached an average weight of 298 grams at about 800 days. This was 44 grams, or 17.3 per cent heavier than the average maximum of all the virgins. Since the average age at birth of the first litter was 136 days, the more rapid increase in weight was partly due to some of the animals being pregnant when the weights were taken. But this is not all. The average age at which the last litter was born was 454 days. At this age the average weight was approximately 275 grams. The growth continued at a gradually decreasing rate until the maximum was reached at the age of about 800 days. The differ-

ence in weights cannot therefore be attributed wholly to the intra-uterine young but to a large extent to sexual activity serving in some manner as a stimulus to increased weight by the mother.

When the growth of those in revolving cages in this group was compared with that of the stationary cages a very marked difference was noted. This difference was the reverse to that seen in the virgins. The average number of litters per rat in the revolving cages was 2.6 and the young 12.6 per rat. In the stationary cages the litters averaged 2.75 and the young 11.75 per rat. Reproductive activities were therefore almost the same. The only noticeable difference other than that of activity was the age at which reproduction began in the two classes. The stationary cage rats delivered their first litter at the average age of 115 days while the average age at delivery of the first litter in the revolving cages was 153 days. Since similar results and conditions were obtained also in groups C and D one may infer that the early bearing of young is a hindrance to growth. There was also a noticeable difference in the average age at which the maximum weight was reached. Those in the revolving cages attained a maximum weight at approximately 670 days after which there was a gradual decline. Those in the stationary cages reached a heavy weight, due to pregnancy, at about the same age and a slightly heavier weight at the age of 880 days.

Fig. 1. Average curves of body weight of females, group A, virgin rats. Light continuous line, normal females in revolving cages; dotted line, normal females in stationary cages; dash line, hysterectomized animals in stationary cages; heavy continuous line, average curve of all classes.

Fig. 2. Average curves of body weight of female rats, group B, light breeding. Light continuous line, revolving cages; dotted line, stationary cages; heavy continuous line, average both classes.

Fig. 3. Average curves of body weight of female rats, group C, medium breeders. Light continuous line, revolving cages; dotted line, stationary cages; heavy continuous line, average of both classes.

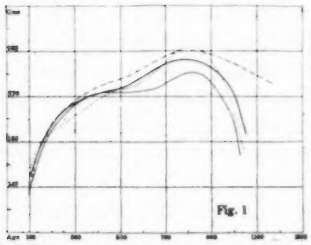
Fig. 4. Average curves of body weight of female rats, group D, heavy breeders. Light continuous line, revolving cages; dotted line, stationary cages; heavy continuous line average both classes.

Fig. 5. Average curves of body weight of hysterectomized rats, group E, excessive sexual indulgence with no reproduction. Continuous line, hysterectomized virgin controls; dotted line, excessive indulgence.

Fig. 6. Average curves of body weight representing each of the five groups of female rats. Light line, group A, virgins; dotted line, group B, light breeders; dash line, group C, medium breeders; dot and dash line, group D, heavy breeders; heavy continuous line, group E, excessive coitus without reproduction.

Fig. 7. Average curves of body weight representing each of the five groups of male rats. Light continuous line, group A, bachelors; dotted line, group B, light breeders; dash line, group C, medium breeders; dot and dash line, group D, heavy breeders; heavy continuous line, group E, excessive sexual indulgence.

Fig. 8. Average curves of body weight of males. Continuous line, group A, bachelor controls; dotted line, average weight of groups B, C, D and E.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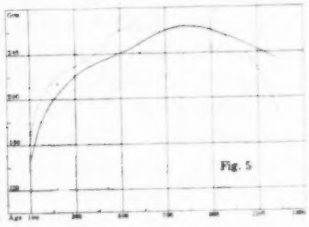
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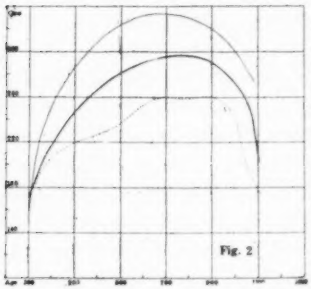
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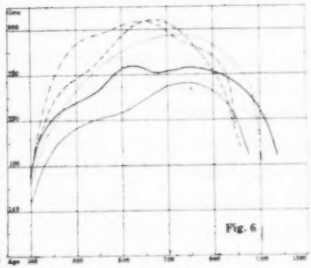
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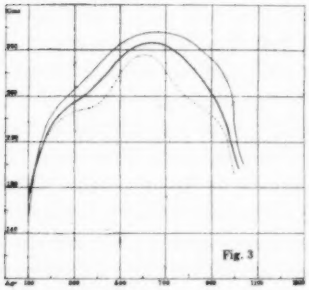
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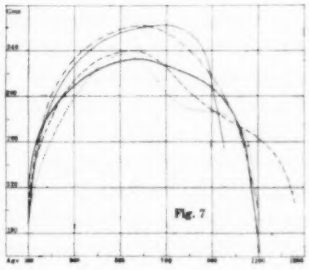
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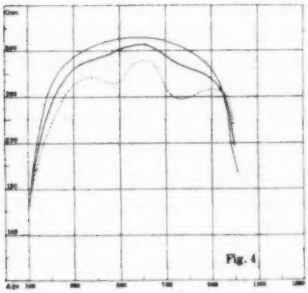
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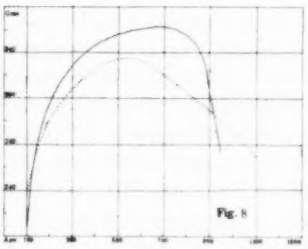
Fig. 8

Females, group C. This group of medium breeders consisted of five females in revolving cages and four in stationary cages. The experiment started when the animals in the revolving cages averaged 78 days of age and 115 grams in weight. Those in stationary cages averaged 75 days of age and 103 grams in weight. The first litters were born when the mothers were at the average age of 171 and 116 days and the last litters at 540 and 641 days respectively. The average number of litters and young born by each female in the revolving cages was 6.5 and 44.2 respectively; in the stationary cages they were 11.5 and 61. This difference was due to the constant presence of the males in the stationary cages which permitted mating at the first oestrus following lactation, while with the others mating trials often failed and were never attempted until the cycles appeared in the activity curves. Figure 3 shows that the difference in growth between the active and inactive groups was not so great as in the light breeders but is quite marked. Like the former group the rats in the revolving cages surpassed in weight those in the stationary cages. The maximum average weight for the group was 306 grams, surpassing that of the light breeders by eight grams and that of the virgins by fifty-six grams. This average maximum weight occurred at about 650 days which was considerably earlier than in either the light breeders or the virgins.

Females, group D. This group of nine rats, five in revolving cages and four in stationary cages, were heavy breeders. That is, the young were destroyed at birth and the mothers remated as soon as possible. The male mates were constantly with the females except two or three days just before the delivery of young. This group was started at the average age of 71 days at which time the average weight was 99 grams. They had the greatest average weight of 304 grams at approximately 600 days of age. Figure 4 shows that the weight increased more rapidly than in the last group of medium breeders and also averaged heavier during their life. The first litter was born at the average age of 131 days and the last at 440 days. The first litter in the revolving cages was born at the average age of 146 days and in the stationary 113 days. The last litters were delivered at the average ages of 427 and 455 days respectively. The heavier weight of the group in the revolving cages seen in groups B and C also occurs in this group. Likewise, the earlier delivery of the first litter by the stationary cage group corresponds to similar conditions in the other groups having sexual indulgence. This adds further evidence to the assumption that early breeding tends to inhibit maximum growth.

Hysterectomized females, group E. Figure 5 represents in dotted line the average curve of weight of 40 females which had opportunity for coitus at each oestrual cycle and the continuous line of 12 hysterectomized virgin control rats. It is readily seen that those having excessive sexual indulgence grew more rapidly and reached a slightly greater weight at about

550 days of age than the controls. They all showed the increase of weight after the menopause found in the other groups. The maximum weight of 266 grams which was reached by both classes, however, corresponds more closely to that of the virgins than to the groups which bore young. The results of this group indicate that excessive coitus without bearing young tends to an increase in growth during the early part of life and to a slightly more rapid decline in weight during senility.

When a comparison of the growth of these five groups of females having different amounts of sexual indulgence was made a marked difference was noted. Figure 6 represents the average weight curve of each of these groups. It shows that the virgins, group A, were the lowest and reached an average maximum weight of 253 grams at approximately 800 days. The hysterectomized group E, with excessive indulgence, were the next in order, reaching a first maximum of 266 grams at 550 days and a second maximum at 800 days. The light breeders, group B, were next and reached a maximum of 298 grams at about 800 days. The medium breeders, group C, and the heavy breeders, group D, were the heaviest reaching a maximum weight of 306 and 304 respectively at about the age of 600 days. The light breeders (B), however, maintained a heavier weight during old age than any of the others. There was a tendency in all groups to show an increased weight after the cessation of the oestrual cycles.

That the greater weight attained by the breeding rats was not wholly due to a greater deposition of adipose tissue or an enlargement of the reproductive organs was shown by the measurements of body and tail lengths taken at the time of autopsies. These showed that there was an increase in stature as well as in weight. The breeding females had an average body length of 186 mm. and a tail length of 177 mm. Corresponding measurements of the virgins were 182 and 166 mm. The hysterectomized rats which had excessive coitus were approximately midway between the above groups. They had an average body length of 184 mm. and a tail length of 172 mm.

The heavier weight of the breeding females and the general tendency of all the females to increase in weight after the menopause may be explained by the results of the investigation of Bugbee and Simond (1926) on the effects of injections of ovarian follicular hormone on body growth. They found that the injection of this hormone caused a loss of weight in young normal and ovariectomized female rats and also in male rats. In studying the effect of injections of the follicular hormone in old female rats we found (Slonaker, 1927) that the majority of the animals showed a reduction in food consumption and spontaneous activity accompanied by an increase in body weight. This effect was of short duration, lasting but a few days, and was followed by a rapid return to normal conditions. We had accounted for this temporary increase in weight as due to the reduction in activity.

If the presence of the follicular hormone tends to inhibit growth in young animals the breeding females had this restraint almost wholly if not completely removed during gestation and a part of lactation. The virgins, however, were under the influence of this hormone during their entire sexual life. This would account in part at least for the differences in growth between the breeding females and the virgins.

The increased weight of hysterectomized rats having excessive sexual indulgence, group E, over the hysterectomized virgin controls might be accounted for in a similar manner. Experiments now in progress have shown that pseudopregnancy can be induced in hysterectomized rats by mating with a normal male. During this condition oestrual cycles are absent and the influence of the hormone wanting. Doubtless many cases of pseudopregnancy occurred in this group which would remove the inhibiting influence and allow an increased growth. After the menopause when there is no longer any hormone liberated, the secondary increase in body weight which all had a tendency to show would be accounted for in a similar manner.

When the similar groups of males are considered we do not find such wide differences in growth weights as was exhibited by the females. Figure 7 shows the average weight curve of each of the male groups. This shows that the bachelors (A) reached the greatest weight of 363 grams at approximately 700 days of age, after this they lost weight rapidly. The heavy breeders (D) were slightly heavier than the non-breeders for the first 600 days and reached their maximum weight of 362 grams at 570 days. After this maximum the loss in weight was more gradual. The remaining groups reached their average maximum weights at about the same age as the heavy breeders. The medium breeders (C) reached 340 grams, the light breeders (B) were next at 338 grams, and the excessive breeders (E) were the lightest, attaining a maximum weight of 333 grams.

If the average weight curve of all the breeding males is compared with that of the non-breeding ones quite a difference is seen both in the weight attained and in the age at which the maximum was reached. This is shown in figure 8. The non-breeders were 27 grams heavier at approximately 150 days later in life. With the exception of a short time at the beginning the bachelors were heavier throughout life and were also slightly heavier at death than any of the breeding groups.

SUMMARY

When albino rats were allowed different amounts of sexual indulgence the following results in growth were evident:

1. The order of growth of the females, as indicated by the average maximum body weight in the five groups from the lowest to the greatest was 1, group A, virgins, 254 grams; 2, group E, excessive mating, no breeding,

266 grams; 3, group B, light breeders, 298 grams; 4, group D, heavy breeders, 304 grams; and 5, group C, medium breeders, 306 grams.

2. The order of growth of the males, as indicated by the average maximum body weight from the lowest to the highest was 1, group E, excessive indulgence, 333 grams; 2, group B, light indulgence, 338 grams; 3, group C, medium indulgence, 340 grams, 4, group D, heavy indulgence, 362 grams; and 5, group A, bachelors, 363 grams.

3. The females showed greater differences in weight than the males.

4. All groups of females showed a tendency to increase weight after the menopause.

5. An increase in weight in the females is associated with sexual indulgence and a decrease in the amount of follicular hormone.

6. Rats having sexual indulgence show a tendency to grow more rapidly during early life than abstainers.

7. The bearing of young at an early age appears to have inhibited body growth in later life.

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THE VALUE OF INTRAVENOUS AND INTRAPERITONEAL ADMINISTRATION OF OXYGEN

GEOFFREY BOURNE[1] AND RALPH G. SMITH[2]

From the Departments of Physiology and Pharmacology, Washington University School of Medicine, Saint Louis

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The effective administration of oxygen to human beings is a matter of considerable difficulty. The old-fashioned funnel and tube method is useless, and although the intranasal catheter (Bourne, 1922) method is of some value it is less efficient than either the oxygen tent (Barach, 1926) or the oxygen chamber (Barcroft et. al, 1918). The former of these is handicapped and the latter rendered practically inapplicable, as regards medical practice outside of institutions, by the complexity of the apparatus required. A difficulty other than that of technique is present in certain patients to whom it is desirable to administer oxygen. Edema of the alveolar walls is not infrequently of a degree sufficient to prevent an adequate diffusion, when the intra-alveolar tension of oxygen would otherwise allow of it.

For these two reasons a number of attempts have been made to evolve other methods of administration that would be both simple and effective. The present investigation was undertaken in order to obtain evidence as to the value of the intravenous and intraperitoneal routes. Anoxemia was produced experimentally in dogs and the variations in the arterial oxygen content effected by these methods were observed.

Previous observations. Although earlier work had dealt with the subject (Nysten, 1811; Demarquay, 1866), it was not until 1902 that a detailed account was made as to the physiological effects of administering oxygen intravenously. Gaertner (1902) made experiments upon a series of dogs. The oxygen was administered in considerable amounts, upwards of 1 cc. per kilo of body weight per minute. The changes noticed were a fall in systolic blood pressure and cardiac irregularity when the dose was in the neighborhood of 2 cc. per kilo per minute. There is no mention of respiratory movements, and no estimation of the oxygen content of the blood before or after. The administration was continuous. The chief conclusion reached was that the adminis-

[1] Fellow of the British Medical Research Council, Rockefeller Travelling Fellowship.

[2] Fellow in Medicine of the National Research Council, U. S. A.

tration of considerable quantities of oxygen by the intravenou route was compatible with life. Stuertz in 1904 gave oxygen to a dog of 15 kilos body weight at a rate of 3 cc. per kilo per minute for two hours. Here again cardiac dilatation, cardiac irregularities and a systolic splashing sound in the heart occurred. Death followed the injection of a larger quantity. No blood gas estimations were made. Tunnicliffe and Stebbing in 1916 gave small quantities of oxygen intravenously to three patients. They report the presence occasionally of a churning sound over the precordium, and state that the clinical condition was improved in two cases. The amount given however was 10 to 20 cc. per minute for periods of 10 to 15 minutes divided by pauses of 2 to 3 minutes, and it is difficult to imagine how such a quantity could have much effect in raising the arterial oxygen content, the normal requirement for an adult being in the neighborhood of 400 cc. per minute. They lay special emphasis upon the dangers of cardiac dilatation. Torraca (1922) gives a brief account of the morbid anatomical findings after the intravenous injection of considerable quantities to guinea pigs; he particularly stresses the tendency for the gas to become liberated in the peritoneal cavity.

Summary of past observations. Oxygen can be safely administered intravenously to animals in quantities of between 1 and 2 cc. per kilo of body weight, and has been administered to man in doses of about 0.25 cc. per kilo of body weight. The untoward results are cardiac irregularity and a fall in systolic blood pressure. There is no mention of the effect of such measures upon the oxygen content of the arterial blood in animals or in human beings. Theoretically the administration of 1 cc. per kilo of body weight per minute, the normal total requirement being about 7 cc. per kilo of body weight, should raise the oxygen tension to normal if the anoxemia is of a degree of about 1 vol. per cent, that is, providing the oxygen so administered is readily and immediately available to the red blood cells.

The present investigation had as its object the determination of the changes occurring in the oxygen content of the arterial blood resulting from the intravenous injection of measured quantities of oxygen. During the experiments several additional determinations were made after intraperitoneal injection in addition.

Method. The dogs used were anesthetized with paraldehyde and ether or with morphine and ether. The blood was withdrawn by arterial puncture from the femoral artery and the oxygen administered steadily into the femoral vein in measured quantity per minute and under a pressure of from 20 to 40 cm. of water. The oxygen content of the arterial blood was measured by the constant pressure apparatus of Van Slyke (1921).

Anoxemia. The experimental production of a reasonably constant degree of anoxemia is a matter of some difficulty since, of the three variables

concerned, respiration rate and depth, circulation rate, and the oxygen content of the inspired air, though it is possible to control the first and third, the second cannot be regulated. In the first series of experiments the animal was allowed to breathe naturally but the air was administered from a large spirometer containing air with a lowered percentage of oxygen. It was found that an oxygen content of about 11 to 12 per cent would produce a definite, constant and a not too severe degree of anoxemia in the anesthetized animal. During the course of a long experiment, however, changes were apt to occur in the sensitivity of the respiratory centre and in the depth of respiration. Nevertheless clear evidence was obtained upon certain points. The second series of experiments was made under artificial respiration of a depth and rate that was found by blood analysis at the time to produce a constant anoxemia. Here the automatic valve and compressed air system of artificial respiration was used, the pressure being kept constant by the interposition of two constant pressure tanks placed in series. With the first two variables held constant the third when it did vary was apt to be subject to more violent change, there being no elasticity. The results obtained differed in hardly any particular from those of the previous series.

Experiments. We first performed a preliminary experiment to determine the quantity of oxygen that could be given safely to an anoxemic dog. The oxygen content of the arterial blood at the beginning of the administration was in each case in the neighborhood of 17.5 vols. per cent. The gas was given for periods of five minutes in quantities increasing by $2\frac{1}{2}$ cc. per minute. A continuous blood pressure record was taken.

The results of this experiment were as follows: Oxygen can be given intravenously in quantities of up to 3 cc. per kilo of body weight per minute for five minutes without causing death. In amounts larger than 0.75 cc. per kilo per minute attacks of acute dyspnea may occur. These are of abrupt onset, the respiratory movements being maximal from the first. There is no gradually increasing embarrassment as might be expected to occur in a progressive heart failure. They vary in severity with the quantity of gas administered, and are accompanied by a fall in systemic blood pressure. They are probably due in the smaller doses to pulmonary embolism, but with larger quantities are doubtless accentuated by an accumulation of gas in the right side of the heart. That this is so is evident from the churning and splashing sounds that accompany systole. These findings as regards changes in blood pressure and of dyspnea were borne out by subsequent experiments and are in accord with previous experimental work; no further reference to them will be made as the oxygen content of the arterial blood is the only safe guide to the value of the results from the clinical point of view. It is thus evident that the introduction of oxygen in quantities sufficient theoretically to raise the oxygen tension of

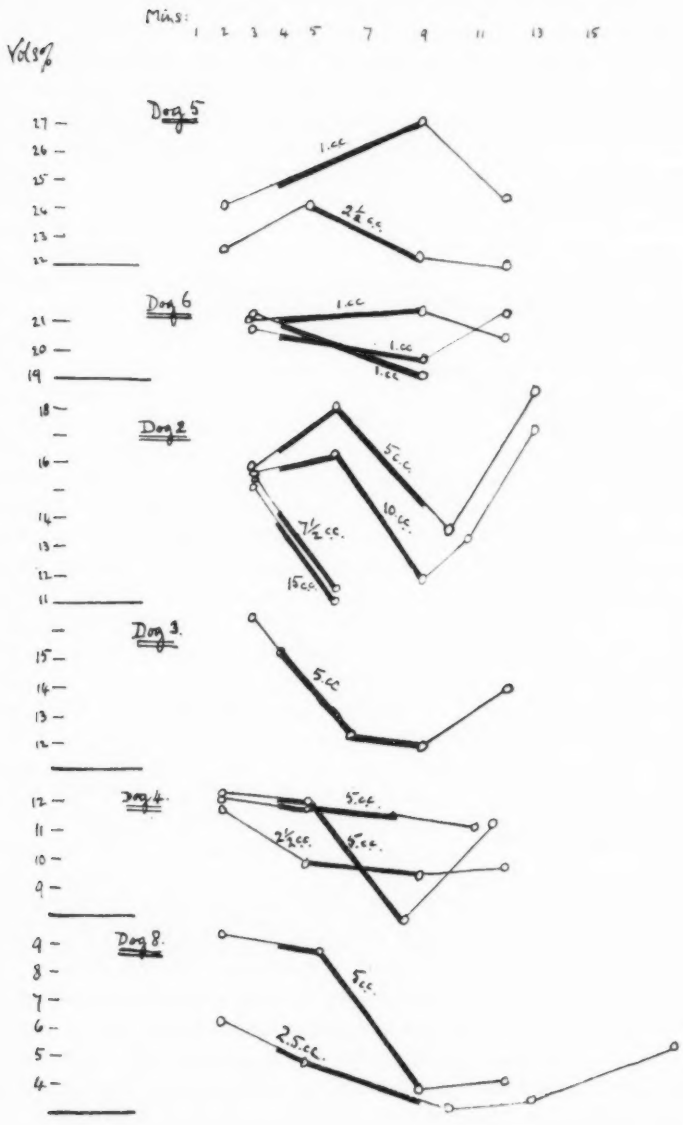

Fig. 1. Determinations of arterial oxygen in volumes per cent after intravenous oxygen. (The thick line indicates the period of oxygen administration, and the figures the dosage per minute. The experiments are plotted separately.)

the arterial blood is a feasible measure. Figure 1 shows the effects upon the arterial oxygen in the subsequent experiments.

It is thus apparent that in quantities which are theoretically sufficient to produce some alleviation of an arterial anoxemia, the intravenous injection of oxygen is followed, presumably as a result of gas embolism, by an accentuation of the oxygen deficiency. This accentuation is more marked in the more severe grades of anoxemia.

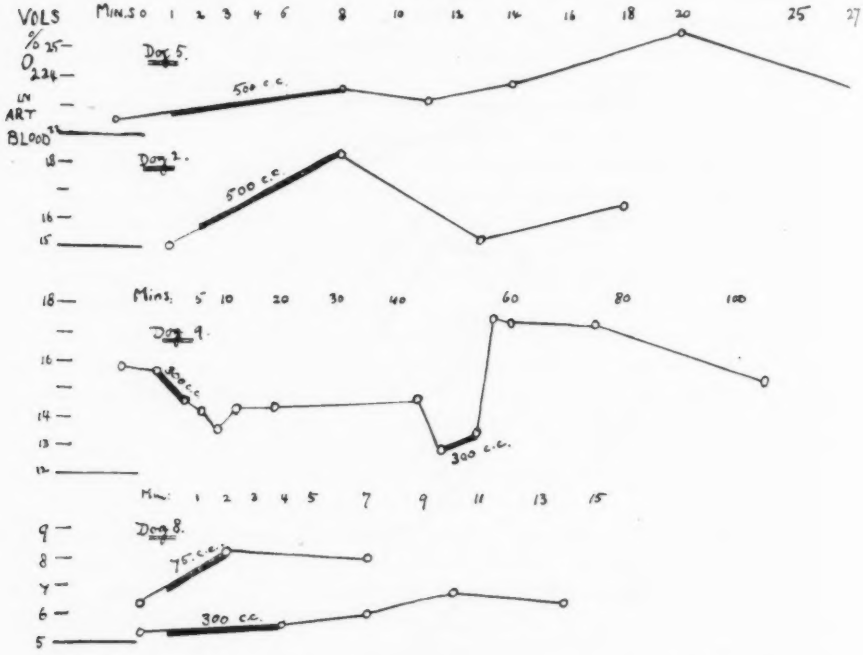

Fig. 2. Determinations of arterial oxygen in volumes per cent after intraperitoneal oxygen administration. (The thick line indicates the period of oxygen administration, and the figures the dosage. The experiments are plotted separately.)

Intraperitoneal administration of oxygen. During the course of the above experiments a few similar investigations were carried out to determine the effect of intraperitoneal injection of the gas (fig. 2). The dogs were in all cases at first rendered anoxemic; this in the case of dogs 2, 4, 5 and 7 was by breathing an atmosphere containing 12 per cent of oxygen, and in the case of dogs 8, 9 and 10 by a just inadequate degree of artificial respiration.

Subcutaneous injection. In dog 9 the subcutaneous injection of 120 cc.

of oxygen in 2 doses 13 minutes apart was followed by no change in the arterial oxygen content.

Discussion. The two conditions necessary for the mobilisation of oxygen in the body are rapid absorption of the gas on the part of the blood and transference of it as oxyhemoglobin. Rapid absorption can only be obtained if a sufficient surface of reduced hemoglobin is presented to an adequate quantity of oxygen at a reasonable partial pressure. The anatomical structure of the lungs enables this to occur when the channel of approach of the gas is normal. Intravenous injection of oxygen at the proper rate, provided no mechanical difficulties were simultaneously set out would seem theoretically to be the best method of attaining this object. It can be conceived that the oxygen would be carried to the lungs and there dispersed over a wide area of pulmonary tissue, whence, being already upon the right side of the capillary walls, absorption would be rapid and in the normal position in the body. It is seen however that, in amounts that would provide some hope of the supply of an adequate amount, pulmonary embolism and right heart insufficiency produce an even greater degree of anoxemia than that already in existence. The determining factor is presumably the size of the bubbles of gas. If the oxygen could be introduced in the form of an emulsion sufficiently fine to pass the pulmonary capillaries there might be some hope of evolving a useful intravenous method of administration. Even so the introduction of such a quantity of fluid into the body would present a difficulty of some magnitude.

Absorption of oxygen from the peritoneal cavity is followed by a rise in some cases in the oxygen content of the arterial blood. This is most marked when the existing anoxemia is not too great. In the case of dog 9 there is no adequate explanation of the fact that only the second of two injections was followed by a rise. In the case of dog 7 the second injection was followed by no change in the oxygen content. It would seem from these experiments that intraperitoneal administration of oxygen if adequately controlled by arterial puncture and blood gas analysis might be a measure worthy of clinical experiment; it is probable that clearer evidence would be obtained since an anoxemia that has been present for some days or weeks is less likely to be subject to the variations that cannot be eliminated in acute animal experiment. Congenital cardiac disease would provide the clearest field for experiment.

CONCLUSIONS

1. Experiments have been performed on anoxemic dogs to determine the effect upon the arterial oxygen content of the intravenous administration of oxygen.

2. In quantities theoretically sufficient to alleviate the anoxemia pul-

monary embolism and right heart failure were probably the factors producing an increase in the condition.

3. Absorption of oxygen from the peritoneal cavity apparently occurs in some cases at a rate sufficient to cause a diminution in an existing anoxemia.

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THE EFFECT OF OVARIAN TRAUMATIZATION ON THE SPONTANEOUS ACTIVITY AND GENITAL TRACT OF THE ALBINO RAT, CORRELATED WITH A HISTOLOGICAL STUDY OF THE OVARIES

GING H. WANG AND ALAN F. GUTTMACHER

From The Psychobiological Laboratory and The Department of Obstetrics, The Johns Hopkins Medical School

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The daily spontaneous activity of a female rat measured by the revolving-drum method shows a regular rhythm, as demonstrated by Wang (1923), Slonaker (1924) (1925), Durrant (1925) and Bugbee and Simond (1926). Every fourth day the animal may run in the drum as much as ten miles or more within the twenty-four hours, while on the other three days it is much less active. This rhythm has been definitely correlated by the use of vaginal smears with the ovulation cycle, the activity being highest during oestrus when the smear shows only cornified epithelial cells. After total ovariectomy, the oestrous rhythm drops out and the vaginal epithelium no longer undergoes the regular cyclic changes (Long and Evans, 1922a). And simultaneously with this physiological effect, the activity shows a 65 to 95 per cent decrease from the pre-operative level and the activity rhythm is permanently suspended.

These observations have been confirmed in more than a hundred spayed rats used during the past five years for different behavior studies in this laboratory. In two other animals, however, in which an attempt was made to remove the ovaries alone without injuring the Fallopian tubes, an entirely different situation was encountered. For a few days immediately after the operation these two rats showed the usual castration effects in both the activity and the vaginal smears; then the activity began to increase rapidly and soon reached the pre-operative average with arrhythmic variations (see fig. 1). Both of these animals subsequently showed several periods of more than twenty days when their activity, although arrhythmic, approximated their normal level. During these periods only cornified epithelial cells were present in the vaginal smears. These findings suggested the possibility that the ovariectomy was incomplete. Moreover, during necropsy small nodules which looked like ovarian tissue were found in both animals at the tip of one uterine horn, but unfortunately these nodules were lost when the specimens were prepared for microscopic ex-

amination. The uteri were sectioned, however, and gave further evidence that the operation had been incomplete in that they had not atrophied; such an effect has always been known to follow complete ovariectomy. The results of these two experiments prompted us to make the present investigation.

In the first place, we wished to determine whether these unusual changes in the activity and the vaginal smears could be repeated in a larger group of rats, the ovaries of which had been definitely injured instead of totally removed. In the second place, we wished to correlate these changes in the activity and the vaginal smears with possible changes in the histological structure of the traumatized glands. As this work proceeded, some insight was gained incidentally as to what part of the ovary produces the hormone which causes the enormous increase in activity and the cornification of the vaginal epithelium during the period of "heat."

METHODS. The method for measuring the activity and the general experimental procedure, which have been reported in previous papers from this laboratory (Richter, 1926; Richter and Wang, 1926; Wang, 1923; Wang, Richter and Guttmacher, 1925) need not be repeated here. Traumatization operations were performed on rats at different ages, but only after each animal had shown a normal activity curve for more than twenty days. The conditions were simplified by the complete removal of one ovary, since we knew from previous experiments that this operation has no effect whatever on either the rhythmic activity or the vaginal smear (unpublished experiments). The remaining ovary was traumatized as follows: By the direct method, either the ovary was almost completely transected by 4 or 5 parallel incisions, or two-thirds of it was removed. Two indirect methods were also used whereby attempts were made to interfere with the ovarian blood supply. The ovary of the rat receives blood mainly from

Fig. 1. Activity and body-weight curves of an incompletely ovariectomized female rat (no. 23). The shaded areas indicate the days when only cornified epithelial cells were found in the vaginal smear.

Fig. 2. *a*. Activity and body-weight curves of a group II animal (no. 203). The first arrow indicates the time when the right ovary was removed and the left ovary was stripped of its blood and nerve supply; the second arrow, the time when the injured left ovary was removed.

b. Activity and body-weight curves of a group III *a* animal (no. 207). The first arrow shows the time when the right ovary was removed and the left stripped of its blood and nerve supply. The second arrow indicates the removal of the traumatized left ovary. The shaded area shows the length of time during which only cornified epithelial cells were found in the daily vaginal smears.

c. Activity and body-weight curves of a group III *b* animal (no. 199). On the day indicated by the first arrow, the right ovary was removed and the left cut through by several parallel incisions. The second arrow denotes the time of the second operation for the removal of the traumatized ovary. The shaded area indicates the length of time during which the daily vaginal smears showed only cornified epithelial cells.

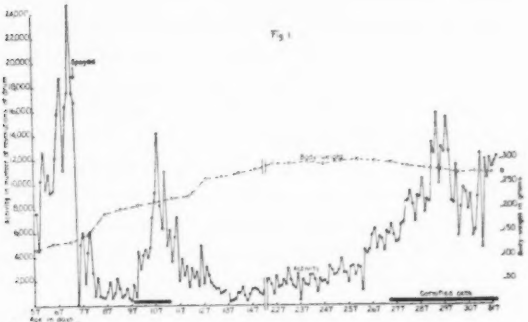
Fig 1
Body weight
Activity
Cornified cells

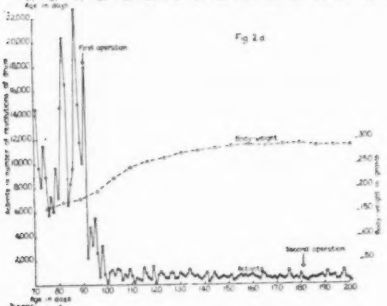
Fig 2a
First operation
Body weight
Second operation
Activity

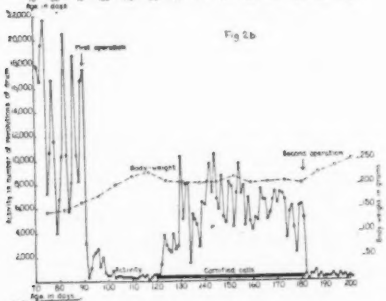
Fig 2b
First operation
Second operation
Body weight
Activity
Cornified cells

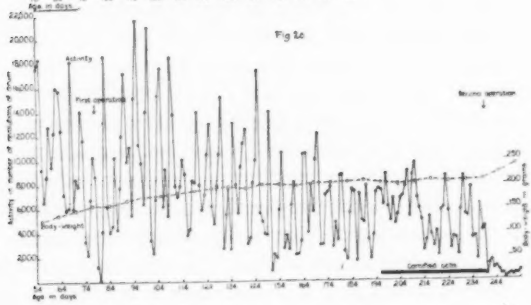
Fig 2c
Activity
First operation
Second operation
Body weight
Cornified cells

the uterine artery, which runs along the uterine mesentery and sends branches to both the ovary and the Fallopian tube. In our efforts to interfere with the blood supply, therefore, we first cleared away all of the fat and connective tissue surrounding the peri-ovarial sac to make sure that blood could not reach the gland except through the branch of the uterine artery. Then, either this artery was tied off by a ligature placed at the tip of the uterine horn, or a small piece of uterine-mesentery was removed at the junction between the oviduct and the cornu uteri. With both of these indirect methods of ovarian traumatization, the nerve supply was also interrupted. Of the four methods the last one was most effective (see table 1).

RESULTS. A. *Changes in activity and vaginal smears.* The immediate effect of the operation was expressed in most of the animals by a marked decrease in activity to the castrate level, the disappearance of cyclic changes in the activity and vaginal smears, and a large increase in body-weight. These conditions persisted from one to four weeks, but then the

TABLE 1

METHOD OF TRAUMATIZATION	GROUP I	GROUP II	GROUP III
Ovary completely transected by parallel incisions	4	0	3
Two-thirds of ovary excised	3	0	2
Ovarian blood supply interrupted by ligature of uterine artery	1	2	0
Ovarian blood supply interrupted by excision of uterine mesentery at junction of oviduct and cornu uteri	0	2	11

picture varied so that the twenty-eight animals fell into three groups. The number of individuals in each group and the method of traumatization used in each individual, are presented in table 1.

Group I. Of all the animals, eight were affected very little or not at all by the operation, quickly regaining their normal running level and body weight, and their cyclic activity and smear variations. This normal picture remained until the traumatized ovary was removed in a second operation, when the usual castrate effects were produced.

Group II. In four rats the running average after the operation was very low and the normal four day activity rhythm was absent. The daily vaginal smears showed none of the normal cellular variations, but presented continuously the di-oestrous phase, with numerous leucocytes and a few nucleated epithelial cells. The body weight continued to increase until it reached the level of the male. The complete records of one of these animals are given in figure 2*a*. The rats of this group, then, showed

the same changes in activity, vaginal smears, and body weight after ovarian traumatization that they would have shown had their ovaries been completely removed.

Group III. The sixteen animals remaining can, with one exception, be grouped in two classes: *a*. In nine, the activity increased rapidly after the initial post-operative drop until it almost reached the running averages of the pre-operative period, and it remained at this new high level with very little daily variation and no regular rhythm. Thus, the high peaks were much lower than those of the pre-operative period, but the averages were approximately the same. Simultaneously with the increase in activity, the vaginal epithelium began to show a consistent cornification (fig. 2 *b*). *b*. Five animals regained not only the normal activity level, but also the activity rhythm and the normal cyclical smear changes soon after the traumatization, and a sixth individual presented a normal picture immediately afterwards. In all six this period of normal activity and normal smears lasted from 30 to 120 days. Then the rhythm suddenly disappeared without much change in the running average; at the same time only cornified epithelial cells were found on the vaginal mucosa (fig. 2, *c*). In all of these fifteen rats the period of high arrhythmic activity and consistent cornification of the vaginal epithelium persisted from 40 to 170 days. Then the animals were either sacrificed for necropsy or reoperated for the removal of the traumatized ovary. Always when a second operation was performed, the usual castration effects were observed in an enormous decrease of daily activity and the consistent presence in the vaginal smears of a large number of leucocytes with a few nucleated epithelial cells (see fig. 2 *b* and *c*).

TABLE 2

GROUP	ACTIVITY		VAGINAL SMEAR
	Level	Rhythm	
I	High	Present	*Normal*—regular oestrus rhythm present
II	Low	Absent	*Castrate—leucocytes and a few nucleated epithelial cells continuously present*
III	High	Absent	*Oestrous*—cornified epithelial cells only continuously present

The sixteenth animal of group III fell into neither of the two subclasses. After the traumatization operation its activity showed a permanent decrease to the level of a spayed animal, although there was a period of consistent cornification in the vaginal epithelium for 120 days and the body weight curve was similar to that of a normal female.

The changes in the activity and the vaginal smears observed from these three groups of rats are best summarized according to table 2.

B. *Macroscopic findings in the genital tract.* From table 2 it would be expected *a*, that the ovaries and the other internal sex organs of the animals in group I had remained normal; *b*, that the ovaries of the group II animals had degenerated and the other internal sex organs had atrophied; and *c*, that some unusual changes must have occurred in the internal sex organs of the animals in group III. These predictions were confirmed by macroscopic examinations made on the twenty-eight animals either during necropsy or during the second operation for the removal of the traumatized ovary. These examinations, made from 100 to 200 days after the traumatization operation was performed, disclosed nothing abnormal in the genital tract of the group I rats except the absence or the distention of the oviduct in a few cases. In the animals of the second group, no ovarian tissue could be found and the uteri were all atrophied. In the fifteen animals of the two sub-classes of group III the ovaries contained exceedingly large follicles, the Fallopian tubes were either distended or absent, and the uteri were greatly hypertrophied and distended with fluid to several times the normal size. In the one remaining animal which showed a permanent post-operative depression in activity the oviduct was absent and the uterus greatly hypertrophied and distended, but the ovary did not seem to contain any follicles. It is important to notice that the ovarian remnants in all of the animals in group III were always much smaller than those of the rats in group I. After these macroscopic examinations the upper end of the uterus together with the tissue above it was removed and preserved in Bouin solution for further histological study.

C. *Histological changes in the uterus.* After the removed tissues had hardened in the Bouin fluid, they were dehydrated, imbedded in paraffin, cut serially from 6 to 10 micra, and stained either with hemotoxylin and eosin or with iron hemotoxylin. These sections were then examined for histological changes in the uterus and the ovary.

In the normal adult female, the uterus undergoes cyclic variations which are dependent on the ovarian rhythm. At the time of oestrus, the uterus is highly distended (about 5 mm. in diameter) by an accumulation of clear thin fluid. The uterine walls are greatly thinned by this distention and the epithelium is composed of a layer of cuboidal cells. The dioestrous uterus presents a markedly contrasting picture. The uterine cavity is collapsed to a mere slit, measuring about 2 mm. in width, the walls are relatively much thicker, and the cuboidal oestrous epithelium is converted into a layer of columnar epithelium (Long and Evans, 1922a).

The eight animals of group I, with the oestrous cycle in both smear and activity unaffected by ovarian traumatization, showed the normal cyclic variations of the uterus. Rat 195, which according to both activity and vaginal smear was in "heat" at the time of the necropsy, had the typical oestrous uterus in both the gross and the microscopic picture. (A_1 in fig.

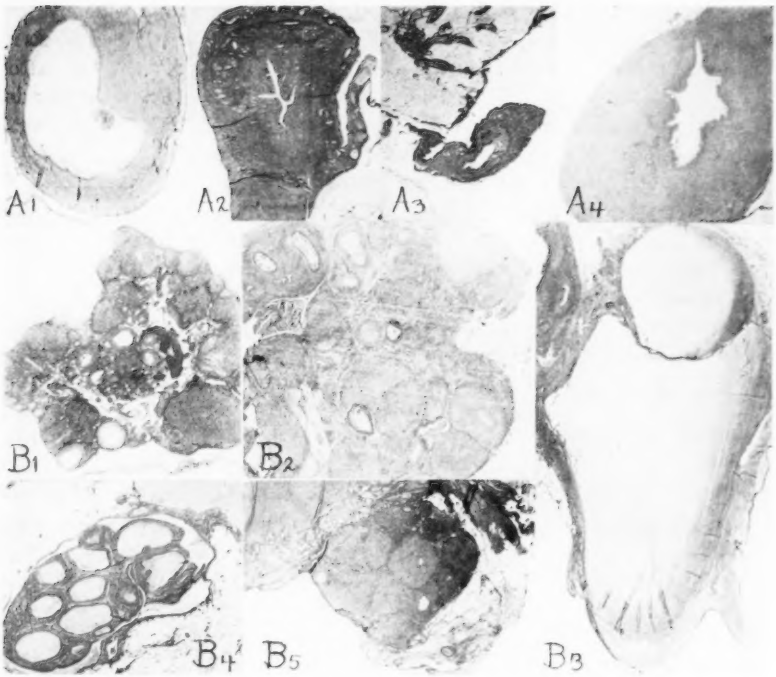

Fig. 3. A. Photomicrographs showing the effects of ovarian traumatization on the uterus.

1. Transverse section of the uterus of rat 195 in group I. Normal oestrous uterus.

2. Transverse section of the uterus of rat 142 in group I. Normal dioestrous uterus.

3. Transverse section of the uterus of rat 201 in group II. Uterus markedly atrophied.

4. Transverse section of the uterus of rat 169 in group III. Uterus somewhat distended and greatly hypertrophied. All these photographs were taken at the same magnification.

B. Photomicrographs showing the changes in the ovary after traumatization.

1. A section of a normal ovary during the dioestrous interval, showing a large number of corpora lutea and Graafian follicles in different stages of development.

2. A section of the traumatized ovary of rat 164 (group I), killed during the dioestrum. Histological structure similar to that in *1*.

3. A section of the traumatized ovary of rat 207 (group III *a*) showing two large follicular cysts, and no corpora lutea.

4. A section of the traumatized ovary of rat 199 (group III *b*), showing a group of small follicular cysts with no corpora lutea.

5. A section of the traumatized ovary of rat 140 (the exception of group III), showing a group of corpora-lutea-like structures with one small follicle. All the photographs were taken at the same magnification.

3). In contrast to this animal is rat 142, whose traumatized ovary was removed during the dioestrum (fig. 3, A_2). The uteri of the four animals in group II whose activity and vaginal smears became castrate-like after ovarian traumatization, showed marked atrophy in the muscles, the glands, and the epithelium (fig. 3, A_3). Entirely different histological changes were found in the uteri of the sixteen animals of group III.

These uteri were similar to the normal oestrous uterus in being distended by fluid and having a large lumen. However, in spite of this distention, their walls were three to four times thicker than those of the normal organ, due to an extensive hypertrophy of the muscular coat. The epithelial lining was composed of a row of high columnar cells which had small basal nuclei and a large amount of cytoplasm, and on many of these epithelial cells there were found finger-like processes which protruded into the uterine cavity. A_4 in figure 3 presents a typical section of these uteri.

D. *The histology of the traumatized ovaries.* In all fifteen animals of group III which showed the high arrhythmic activity and the consistent cornification of the vaginal epithelium following the traumatization of the ovary, decidedly abnormal ovarian tissue was found. On the other hand, in the eight rats of group I, which were so unaffected by the operation that they continued to show a regular four to five day rhythm in activity and smear, the ovarian tissue was indistinguishable from that of normal adult females; microscopic examination revealed normal Graafian follicles in all stages of development, several sets of corpora lutea from different ovulations, and normal interstitial tissue (compare fig. 3, B_1 and B_2). No ovarian tissue could be found in sections of the tissue removed from the four group II animals. Apparently at the traumatization operation the ovary was so extensively injured that it was later completely absorbed and the rats were thus converted into castrates as effectively as if they had been purposely spayed.

The fifteen animals of classes *a* and *b* of group III all presented a very atypical picture. The ovaries of this group were quite dissimilar, in spite of the fact that the animals were very much alike as far as their activity and smears were concerned. One of the most striking features which distinguished these ovaries from normal ones was the scarcity or total absence of corpora lutea. Of the few corpora present in the sections, none was very recent. A few primordial follicles occurred in nine animals. Mature and normal Graafian follicles were found in ten of the series, but they were not as numerous here as they are in the normal adult female. Interstitial cells appeared in twelve, in several instances more abundantly than in normal specimens. The only structure consistently present in all of the fifteen ovaries, was the follicular cyst, and it seems to be the common denominator of group III, associated throughout with the high arrhythmic activity,

Fig. 4. Photomicrographs showing the cellular components of the wall of the follicular cysts, and also those of the corpora-lutea-like structures in the ovary of rat 140.

a. Parts of the walls of two big cysts, showing the compression and reduction of the granulosa layer with the disappearance of theca interna.

b. Part of the wall of a large cyst, showing the complete absence of granulosa and theca interna. The flattened theca externa is the only structure left.

c and *d*. Parts of the walls of two small follicular cysts, showing the existence of the three layers of the normal follicle.

e. Part of the wall of a follicular cyst, showing the luteal-like cells which are frequently found in the wall of the cysts.

f. One of the corpora-lutea-like structures found in the traumatized ovary of rat 140.

These photomicrographs were kindly made for us by Miss Cecilia V. Bisson.

the continuous presence of cornified cells, and the hypertrophy of the uterus (see fig. 3, B_3 and B_1).

The cysts varied greatly in size and number, the largest occupying the greater part of the ovary. All were lined by granulosa cells, the thickness of the layer and the degree of compression depending on the size of the cyst so that in the largest cyst they were compressed to a broken layer of small flat cells. In many of the cysts, especially in the larger ones, the theca interna was absent, the cyst wall being composed only of the thin granulosa, and a layer of flattened theca externa. In several instances the walls showed a circumscribed island of lutein-like cells. It was impossible to say whether these had originated from theca interna or from granulosa cells (see fig. 4 *a*; *b*, *c*, *d* and *e*).

The fragment of ovary found in the animal which showed continuous cornification of the vaginal epithelium and a castrate level of activity, presented still a different picture. This ovary showed no cysts and no large Graafian follicles, only one or two primordial follicles, and very little interstitial tissue. It was made up almost completely of corpora lutea-like structures, the cells of which seemed to be enlarged theca interna cells. And in the center of two of these corpora there was still a cavity which was filled with coagulum (see fig. 3 B_5, fig. 4*f*).

E. *Correlation of the cellular structure of the traumatized ovaries with the changes in the activity and the vaginal smears.* From the facts presented in the last four sections, two conclusions can be drawn: *a*. It is likely that the continuous secretion from the follicular cysts causes the high arrhythmic activity, the consistent cornification of the vaginal epithelium and the hypertrophy of the uterus, since the cyst is the only histological structure which is found consistently in every animal in group III. *b*. This secretion may be produced by the granulosa cells, since these are the only cellular element consistently found in the cysts, which may have secretory functions.

The first conclusion is supported by the observations of Long and Evans (1921), and also by those of Hess (1906), Williams (1909), Albrechtsen (1917) and others. Long and Evans found that out of 800 normal rats, seven only showed consistent cornification of the vaginal epithelium over long periods of time. The ovaries of these animals contained follicular cysts. Hess, Williams, Albrechtsen and other veterinary surgeons have observed that hydrops folliculi are always present in the ovaries of cows, sheep, mares and bitches which have become nymphomaniac and remain in persistent oestrus.

Experiments performed (by A. F. G.) on two guinea pigs in which some ovarian tissue was accidentally left after ovariectomy, gave further support to this conclusion. After the operation the vaginal closure membrane of these two animals remained open continuously and the cells of the vaginal

mucosa were consistently cornified. During necropsy follicular cysts and a markedly hypertrophied uterus were found. One horn of the normal uterus in the guinea pig weighs about 500 mgm. when fresh and about 60 mgm. when completely dried. The wet weight of one horn of the uteri of these two guinea pigs with the traumatized ovary was 1872 and 1963 mgm. respectively, and the dry weight, 295 and 310 mgm.

The second conclusion, that the fluid in the cysts may be produced by granulosa cells, differs from the view of Zondek and Aschheim (1926) (1927). They claim to have proved, by vaginal smears made on spayed mice with parts of human Graafian follicles implanted, that the theca interna cells produce the hormone which causes the oestrous change in both the smear and the behavior. In view of the uncertain success of implantation and the difficulty of separating the different cell layers of the follicle, we are inclined to leave the decision of this problem to further experimentation.

It must be pointed out that it is difficult to understand the results given by the one animal which stands apart in group III. It showed a cornified vaginal epithelium and an hypertrophied uterus with low arrhythmic activity, and its traumatized ovary contained almost nothing but corpora-lutea-like structures, the cells of which seemed to have originated from the theca interna. These peculiar structures looked like those described by Matzuyama (1921) as occurring in the ovaries of some normal rats living in parabiosis with either a male or a female castrate. In these experiments, he found great distention of the uterus associated with either follicular cysts or large corpora-lutea-like structures in the ovary. And he showed the origin of the cells of these structures to be in the layer of theca interna. These results, then, together with our own, seem to support the view of Zondek and Aschheim that it is the theca cells which give out the hormone for the growth of the genital tract. Furthermore, the combined data suggest that the hormone which causes oestrus and the consequent general restlessness is not the one which promotes the growth of the genital tract. However, we must leave these questions open and await the answer in further experimentation.

Discussion. From the results of the above experiments, we see that slight traumatization causes no damage to the ovary and therefore no changes in activity and the vaginal smear, whereas severe injury causes complete absorption of the ovary and produces the usual castration effects on both the vaginal epithelium and the activity. Traumatization of a severity somewhere between these two extremes causes formation of cystic follicles, with subsequent high arrhythmic activity, consistent cornification of the vaginal epithelium, and hypertrophy of the uterus.

After recovery, the moderately traumatized ovary undergoes marked changes. The corpora lutea gradually degenerate, and the Graafian

follicles continue to grow, although the ova disappear entirely. The granulosa cells secrete continuously so that the follicle is distended by accumulated fluid. The increased pressure thus caused brings about the degeneration of the theca interna and the gradual thinning of the layer of granulosa, which also partially degenerates. At a later stage, some of the granulosa cells and the theca interna are transformed into luteal cells. Since the follicle is so much enlarged and the granulosa and the theca interna layers are so much reduced, no corpus luteum is formed. This explains the contrast in the histology of these traumatized ovaries and that of the normal ones. In the injured ovaries it is the follicles and the follicular cysts which dominate the picture, whereas in normal ovaries it is the corpora lutea.

We believe that the oestrus cycle in all animals depends on the periodic secretion of the granulosa cells. In the rat, therefore, the four-day activity rhythm associated with oestrus is ultimately dependent on this cellular secretion which occurs periodically under normal conditions. In the injured ovary, on the other hand, as soon as large follicles are formed, the granulosa cells pour out their secretion without interruption, the animal receives a constant chemical stimulus, and the activity is maintained on a high level with no rhythmic variations. At the same time, through the constant hormonal influence, the growth of the whole genital tract is stimulated: the uterine wall hypertrophies and there is a proliferation of cells in the vaginal epithelium so that the superficial layers slough off continuously and cornified cells are constantly present in the smears.

Two things still remain to be explained: Why do only the moderately injured ovaries show these changes? And how are the cysts formed?

A. One important difference between the macroscopic picture of the slightly and the moderately injured ovaries has been pointed out above,—the moderately injured ovaries are always smaller in size than either the slightly injured or the normal ovary. The mass of ovarian tissue left in the body after traumatization, then, is an important factor in determining whether cysts are formed or not. The smaller the mass, the greater the chance of cystic formation. This view agrees with observations made by veterinary surgeons (Williams, 1909) that follicular cysts always occur when a small piece of ovary is unintentionally left in the body after an attempt at complete ovariectomy.

B. The formation of the follicular cysts seems to depend on the relationship beween the anterior hypophysis and the ovary, demonstrated by the recent work of Philip E. Smith (1926) (1927a, b). He showed that the removal of the anterior hypophysis causes the ovary in the rat to cease functioning, but the function can be restored by daily implantations of anterior pituitary substances. Such implantations cause precocious sex-maturity in immature rats, and the formation of follicular cysts in adults. From these facts it seems likely that there is a delicate balance between

the mass of the ovarian tissue and the amount of the secretion from the anterior hypophysis. The absence or insufficiency of this secretion will suspend or retard the function of the ovary, while increased secretion will produce follicular cysts. The observations of Matzuyama cited above are highly significant in this connection. He found hydrops folliculi with uterine hypertrophy in normal female rats living in parabiosis with either a castrated male or a spayed female. And Smith demonstrated that the anterior hypophyseal hormone can pass through one animal to the other in parabiosis and that in this way the ovarian function of hypophysectomized animals can be restored.

If the proper functioning of the ovary depends on the balance between the mass of ovarian tissue and the amount of anterior hypophyseal hormone secreted, a reduction in the size of the ovary will have the same effect as an increase in the secretion of the anterior pituitary body. As mentioned before, all the traumatized ovaries which showed cystic follicles were much smaller than those which did not. Therefore, the formation of cysts in these ovaries may have resulted from the reaction of a small mass of ovarian tissue to a relatively much larger amount of anterior hypophyseal hormone.

This suggestion also explains the observations made on the ovaries transplanted into castrated males and reported by us in a previous paper (Wang, Richter and Guttmacher, 1925). These transplanted ovaries showed a scarcity of corpora lutea and the presence of follicular cysts. In comparison with normal ovaries, these transplants are by far smaller in size. Hence the occurrence of the follicular cysts in this case also is probably the result of a small mass of sex gland reacting to a large amount of anterior hypophyseal hormone. Finally it is important to note further that, according to Athias (1922), small pieces of ovarian tissue which have survived either auto-transplantation or grafting into male or female castrates, always show cystic follicles.

However, there are some experimental results which are contrary to this suggestion. In the first place, we found in the exceptional animal of group III that hypertrophy of the genital tract with low activity was associated with the presence of corpora-lutea-like structures in the traumatized ovary, follicular cysts being absolutely absent. In the second place, Matzuyama found either large corpora-lutea-like structures or follicular cysts in the ovaries of normal rats living in parabiosis with a castrate. And in both cases, the uterus shows great distention. In the third place, Long and Evans (1922b) found the persistence of corpora lutea and atrophy of the uterus as the result of daily injections of the extract from the anterior pituitary body of cows. At present it is very difficult to harmonize these results with the suggestions made above.

SUMMARY

1. Experiments were performed on the white rat to ascertain *a*, the changes in the activity and the vaginal smears after experimental ovarian traumatization, and *b*, the correlation of these changes with the histological structure of the traumatized ovaries.

2. Out of twenty-eight animals, eight showed no change in either the activity, the vaginal smears, or the cellular structure of the ovary; four showed castrate effects in both the activity and the vaginal smears and complete degeneration of the ovaries; fifteen (53 per cent) showed changes in both the activity and the vaginal smears; and one showed smear changes with a castrate level of activity.

3. The change in activity was expressed in the disappearance of the normal four day oestrous rhythm, despite the maintenance of the pre-operative daily running averages.

4. In the vaginal smears, the normal four day cyclic variations in cellular contents also disappeared, and only cornified epithelial cells were consistently observed. The uterus showed great hypertrophy.

5. These changes in the activity and the vaginal smears lasted until either the traumatized ovary was removed or the animal was killed. We have observed them from 40 to 170 days after the traumatization operation was performed.

6. After the injured ovary had been removed, the usual castration effects on both the activity and the accessory sexual organs were obtained.

7. It is the secretion from the follicular cysts which is responsible for the changes in both the activity and the accessory sexual organs found in rats whose ovaries have been moderately traumatized, since these cysts were the only structures consistently found in the injured ovaries of these animals.

8. The only cellular element constantly present in the follicular cysts was the granulosa layer. Thus, the granulosa cells may have given out the hormone which produced increased activity, cornification in the vaginal epithelium, and hypertrophy of the uterine wall.

9. Suggestions are made regarding the problems of *a*, why only certain injured ovaries undergo cystic formations, and *b*, how the cysts are formed.

10. In one animal consistent cornification of the vaginal epithelium and uterine hypertrophy were found with low arrhythmic activity, and its ovary contained neither follicular cysts nor large Graafian follicles, but only corpora lutea.

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SOME EFFECTS UPON THE YOUNG OF INADEQUATE MATERNAL DIETS

I. Polyneuritis and Hemorrhages

C. ULYSSES MOORE, JESSIE LAIRD BRODIE and ROBERT B. HOPE

From the Collins Nutritional Research Laboratory, University of Oregon Medical School, Portland, Oregon

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Experimental work has now definitely demonstrated that diets adequate in vitamin B for growth and for reproduction are not necessarily adequate for lactation. In our work with kale and spinach using the dried vegetables as the sole source of vitamin B, although the mothers grew normally and had full-sized litters, 63 per cent of the young died during the first four days of life and 30.6 per cent just prior to weaning at twenty to twenty-two days (Moore and Brodie, 1926; 1927a, b). Others working with vitamin B have mentioned somewhat similar results (Hartwell, 1921a, b; 1925; Heller, 1923; Hogan and Harshaw, 1924; Anderegg, 1924; Grant, 1924; Nelson and co-workers, 1923, 1926a, b; Mattill and Stone, 1924; Sure, 1924) but no such investigation has included necropsy findings. It therefore seemed eminently desirable to have a clinico-pathological study made of the young that die before three weeks of age.

In this investigation the diet used consisted of casein 18, crisco 3, cod liver oil 2, salt mixture (# 185 McCollum) 4, dextrin 71 and yeast[1] 2. Upon this ration the growth rate from weaning to sexual maturity was normal. The reproductive and weaning records of forty litters are given in table 1. It is noteworthy that 72.9 per cent of the offspring died before weaning time. About 45 per cent of the mortality occurred before the animals were one week old.

The chief clinical sign occurring during the first week was subcutaneous hemorrhage around the snout, the feet and the glandular adipose tissue between the shoulders. Autopsy on those dying disclosed in addition extensive visceral hemorrhages. The stomachs showed white and expanded through the thinned abdominal walls and autopsy proved them full of curded milk. The mammary secretion therefore was not lacking in quantity.

[1] The yeast used for these tests was powdered yeast foam tablets, a product of the Northwestern Yeast Company of Chicago. Conn. Expt. Sta. Bull. 240, p. 38, reports that this product closely approximates brewer's yeast in vitamin B potency.

TABLE 1

Reproduction and lactation of albino rats

Synthetic diet (basal I*) containing 2 per cent yeast

	NUMBER OF FEMALES	NUMBER OF PREGNANCIES TO DATE	TOTAL YOUNG BORN	AVERAGE SIZE OF LITTER	NUMBER KILLED TO REDUCE LITTER	NUMBER WEANED	NUMBER DYING			TOTAL DEATHS	
							First week	Second week	Third week	Number	Per cent
First generation	10	16	98	6.1	12	24 (28%)	27 (43.9%)	5 (7.8%)	30 (48.3%)	62	72
Second generation	5	5	38	7.6	7	2 (6.4%)	7 (24%)	2 (7%)	20 (69%)	29	93.6
Same diet as above plus 12% lemon juice in drinking water given ad lib.											
First generation	3	6	39	6.5	2	14 (38%)	10 (43%)	None	13 (57%)	23	62
Second generation	7	13	88	6.8	9	23 (29%)	29 (52%)	None	17 (48%)	56	71
Total	25	40	263	6.6	30	63 (27%)	73 (45.7%)	7 (4.3%)	80 (50%)	170	72.9

* Basal diet I: Casein 18 per cent, salt mixture (#185 McCollum) 4 per cent, crisco 3 per cent, cod liver oil 2 per cent, dextrin 71 per cent, yeast 2 per cent.

On this diet reproduction was good but 72.9 per cent of these 233 young died before weaning time.

The animals living twenty to twenty-two days had nearly normal growth curves during the first two weeks (chart 1). Clinically they may be divided into three groups according to their predominant symptoms. The first and largest group had profuse watery stools, marked drop in body temperature, cold extremities and a gradually developing flaccid paralysis with death within three days. The temperatures were too low to be recorded by an ordinary clinical thermometer. A rat's normal temperature averages about 100°F. (37.8°C).

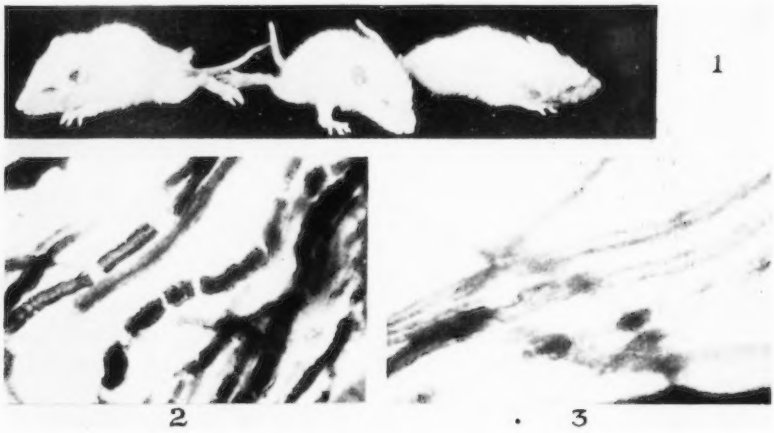

Fig. 1. Young (age 21 days) nursed by female on synthetic diet containing 2 per cent yeast. Note paralysis of hind quarters and general prostration.

Fig. 2. Photomicrograph of teased sciatic nerve (Marchi stain) of an animal dying of paralysis at 21 days. The extensive myelin degeneration is well shown by the beaded fiber in the center of the field. Diet of mother (basal I containing 2 per cent yeast).

Fig. 3. Photomicrograph of teased sciatic nerve (Marchi stain) of an animal 6 weeks after clinical recovery from polyneuritis. The paralysis developed in a nursling at 3 weeks of age. The maternal diet was basal I containing 2 per cent yeast. Note that some fibers still show myelin degeneration.

A second and smaller group showed in addition to the watery stools and subnormal temperature a spastic paralysis and noticeable hyperesthesia. The paralysis usually appeared as an arching of the back (fig. 1) and a stiffening of the gait, the animal either walking on the very tips of its toes or folding the toes under and walking on the dorsum of the front paws. Later characteristic flaccid paralysis occurred.

The third and smallest group was characterized by irritability, hyperesthesia and convulsions. Convulsive attacks were heralded by a shrill

squeal followed frequently by a rapid extension of the hind legs sufficient to throw the animal into an erect posture or make him jump several times his own length. If he struck the side of the cage he would run up and cling there holding on with his teeth until death or exhaustion released him.

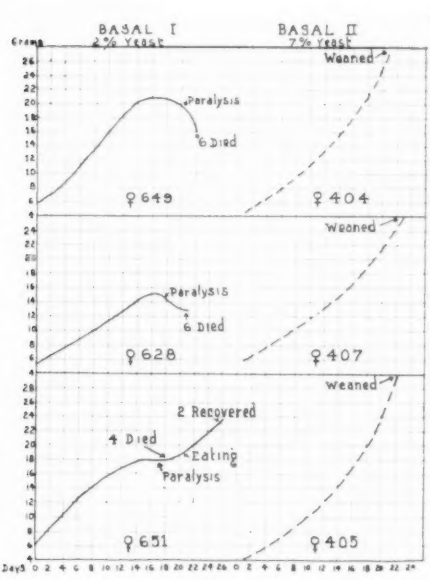

Chart 1

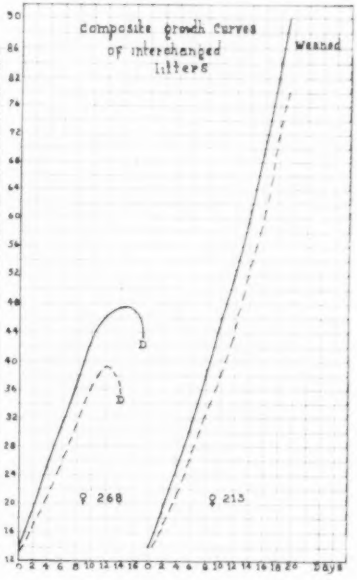

Chart 2

Chart 1. Composite growth curves of litters nursed by mothers on synthetic diets containing 2 per cent (————) and 7 per cent (----------) yeast respectively. The young of mothers on the diet containing 2 per cent yeast developed paralysis during the third week. Death followed in all but two young of female 651. These two were strong enough to eat from the mother's dish and recovered. On the diet containing 7 per cent yeast no paralysis developed and all were successfully weaned.

Chart 2. Each graph represents the sum of the weights of three young. The two curves on the left indicate the growth of the six young suckled by the mother (no. 268) on a synthetic diet, basal I, containing 2 per cent of yeast. The three (----------) born of as well as nursed by her died at 14 days of age, *D*, while the other three (————) nursed by her but born of the stock mother (no. 213) died at 17 days. The two right hand curves show the excellent growth obtained by the six young nursed by the stock mother (no. 213).

To determine whether this paralysis in sucklings was a true polyneuritis the sciatic and other nerves were removed and stained by the osmic acid and Marchi methods. Extensive myelin degeneration was found in every

case (fig. 2). Of the animals living until three weeks of age approximately half were strong enough to eat from their mother's dish and these recovered slowly from their paralysis. A number of these animals were autopsied and considerable myelin degeneration was still present six weeks after clinical recovery (fig. 3).

To test whether the dietary deficiency was both pre- and post-natal, two litters were interchanged. Female 268, a mother raised on the above synthetic diet including 2 per cent yeast, and female 213, raised on the ordinary stock diet (milk, table scraps and McCollum's stock grain mixture), bore on the same day six young each. Immediately after birth three from each litter were interchanged. During the first ten days little difference in growth was noted (chart 2). Then the young nursed by the mother on the synthetic diet began to lose weight and appeared markedly anemic. On the twelfth day all three of the young born of and nursed by this mother developed marked paralysis and died two days later. The following are the salient points in their autopsies:

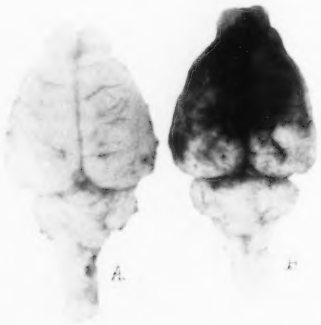

Fig. 4. Photographs of brains of rats two weeks old. A. Normal. B. Cerebral hemorrhages occurring when the maternal diet is deficient in vitamin B.

Large intra-cranial hemorrhages, a characteristic one being shown in figure 4; smaller hemorrhages in liver, lungs, dorsal mesentery and thymus. Brain and cord formless.

On the fifteenth day, just after the deaths noted above, the three young born of the stock mother but nursed by the mother reared on the synthetic diet showed the same type of paralysis and died two days later. Their autopsies disclosed the following pathology:

Extensive hemorrhage over most of both cerebral hemispheres in one: hemorrhages in thoracic and abdominal viscera in all three. In two the thymus was less than half normal size. The sciatics of both groups showed marked myelin degeneration.

In the meantime all six of the young nursed by the mother on the stock ration were successfully weaned. One of those nursed by her but born of the test mother was killed at four weeks of age and autopsied. No pathological condition was found. The remaining five grew to maturity uneventfully. This experiment demonstrates that milk from mothers on an optimum diet may overcome, to some extent, deficient prenatal development; while milk from mothers on a deficient diet may counter balance an optimum prenatal development.

That this hemorrhagic condition is not analogous to that of scurvy is

demonstrated by the fact that the inclusion of 12 per cent lemon juice in the drinking water (table 1) gave no beneficial results. Also the vitamin B content of the added lemon juice (Bacharach, 1925) did not prevent polyneuritis.

To determine the amount of vitamin B needed by lactating mothers one of those on the above synthetic diet was given an additional 5 per cent of yeast two days before the birth of her second litter. The six which she was allowed to nurse out of the ten delivered were carried successfully through the lactation period with no signs of paralysis. After weaning these continued on the diet containing 7 per cent of yeast and showed very normal growth. Their reproductive and lactation histories

TABLE 2

Reproduction and lactation of albino rats on synthetic diet (basal II) containing 7 per cent desiccated yeast*

	NUMBER OF FEMALES	NUMBER OF PREGNANCIES TO DATE	TOTAL YOUNG BORN	AVERAGE SIZE OF LITTER	NUMBER KILLED TO REDUCE LITTER	NUMBER WEANED	NUMBER DYING
First generation	4	6	43	7.1	7	29 (80.6%)	7 (19.4%)
Second generation	6	11	85	7.6	25	58 (96.6%)	2 (3.3%)
Total	10	17	128	7.35	32	87 (90.7%)	9 (9.3%)

On this diet reproduction was better than in table 1 and only 9.3 per cent of these 96 young died before weaning time.

* Same as basal I except dextrin was reduced to 66 per cent and yeast increased to 7 per cent.

Note: The deaths occurring in the first generation were on the first day in one litter only. Subcutaneous hemorrhages marked. The two deaths in the second generation were in a litter where the mother escaped from her cage. Exposure was assigned as the cause.

are given under the first generation of table 2. The only young lost were in the first litter of female 405. They showed hemorrhages at birth similar to those mentioned above. Over 80 per cent of the young of this generation were successfully weaned. In the second generation on this diet only two young, or 3.3 per cent were lost. These deaths were probably due to exposure after their mother escaped from the cage.

Occasionally toward the end of the lactation period a slight dragging of the hind limbs and a tendency toward diarrhea appear, even when the mothers receive 7 per cent of yeast. The animals invariably recover, however, before the symptoms become severe. In these experiments also it was not uncommon to find subcutaneous hemorrhages upon the

newborn. With the exception of the litter noted above these hemorrhages were absorbed in a few days. Apparently this level of yeast is close to the minimum for some nursing mothers. A series of experiments is now under way using higher percentages of yeast, to determine if possible the optimum amount.

On neither 2 per cent nor 7 per cent yeast have we found that the mother tends to sacrifice her own body in order to pass this vitamin on to her young. Although practically 100 per cent of the young nursed by the mothers on the diets containing 2 per cent yeast developed polyneuritis, none of the adults have exhibited any clinical manifestations nor have their sciatics shown any signs of myelin degeneration.

As shown in tables 1 and 2, the average size of the litters from mothers on the 2 per cent yeast is below the average of those on the diet containing 7 per cent yeast. The latter is almost normal for our stock colony. The reduction may have occurred in three ways: a smaller number of ova discharged, a smaller number successfully impregnated, or implanted, or a larger number of absorptions or stillbirths. The stillborn are almost immediately devoured by the mother so that a count can rarely be made. The second generation on 2 per cent yeast shows a higher average litter size but also an enormously higher death rate, indicating a physiological compensation.

A study of chart 1 reveals a striking similarity during the first two weeks between the growth curves of the litters on the different percentages of yeast. It is only when the myelin normally should be fully laid down that the clinical manifestations of the deficiency become apparent and the growth of the animals on the 2 per cent yeast slows up. The consistency of this finding leads us to believe with other workers (Mitchell, 1919; Emmett and co-workers, 1920a, b; Hauge and Carrick, 1926) that the so-called water soluble B vitamin contains both an anti-neuritic and a growth fraction and that the proportion of these two factors varies in different materials. Yeast, from such work as that of Goldberger and Lillie (1926) seems to be deficient in the anti-neuritic vitamin but comparatively rich in the growth factor.

Although the evidence seems fairly conclusive we hesitate to state unreservedly that the intracranial and other hemorrhages are due entirely to vitamin B deficiency. However the definite association of cerebral hemorrhage with an inadequate diet is worthy of careful consideration. The possibility that our diets may be lacking in some other factor is now being investigated.

SUMMARY

1. Diets adequate in vitamin B for the normal growth and reproduction of the mother are not necessarily adequate for the successful nursing of her young.

2. On a diet containing 2 per cent yeast as the sole source of vitamin B the size of the litter was reduced and 73 per cent of the young were lost before the end of the weaning period.

3. One-half of these deaths occurred during the first week. In these cases the predominating pathology consisted of subcutaneous, visceral and intra-cranial hemorrhages. The remaining deaths occurred in the third week with marked paralysis. Autopsy showed myelin degeneration of the sciatics, vagi and other myelinated nerves and numerous macroscopic and microscopic visceral and intra-cranial hemorrhages.

4. The clinical picture during the third week includes marked paralysis, profuse watery stools and a subnormal temperature.

5. The addition of lemon juice to the ration had no visible effect upon either the hemorrhagic condition or the paralysis.

6. An increase of the yeast percentage from 2 to 7 in the mother's diet decreased the mortality in the offspring from 72.9 per cent to 9.3 per cent, practically eliminated the paralysis, and greatly lessened the hemorrhagic condition.

7. The paralytic rats receiving directly the experimental ration containing 2 per cent yeast recovered completely.

8. This work offers evidence in substantiation of the separate identities of a B-growth and an anti-neuritic factor in yeast.

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THE INHIBITION OF ACID IRRITATION BY SUGARS

WALTER R. PENDLETON

From the Hull Physiological Laboratories, University of Chicago

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An irritable area developed in my esophagus a few days after local heat stimulation of the same used in an effort to produce hiccups. While the region was still hypersensitive I drank some juice prepared from rather sour grapes and obtained a marked burning sensation—apparently from the middle third of the esophagus. For the sake of flavor I added about 40 per cent of sucrose to the remaining juice. This made the grape juice most markedly less irritating—perhaps less than one-fourth as irritating as before. The following work arose from a desire to further establish this observation; to analyze the principles involved; to determine why the addition of sugar to fruit juices reduces the acid taste; and to find whether the increased sugar content of oranges and plums, etc., on ripening might be materially responsible for the decreased corrosion of teeth on eating the same, the corrosion being commonly evidenced by a chalk-like, crumbling sensation of the teeth. A study of the underlying principles was made on frogs and then repeated in part on the human. Certain chemical work was done in a further effort to explain the findings.

OBSERVATIONS ON FROGS. Stock solutions were prepared of all acids.[1] The same quantity of the stock solution was measured off for the acid solution (solution I) and the sugar plus acid solution (solution II). A weighed quantity of sugar was added for solution II. The solutions were each made to 50 cc. with distilled water. Ten cubic centimeters of each solution were then titrated with N/10 NaOH using phenolphthalein as indicator. Thus 35 to 40 cc. of each solution remained and this was used in testing the reflex time of the frogs.

A spinal frog was swung by a bent pin through the floor of its mouth and tests were made by immersing one foot to just above the ankle in a solution. The solution would be taken away immediately after a reflex was given and the frog then immersed to the middle of its body in a beaker of water for about 10 seconds. Tap water was generally used for this purpose as its effect was found to be identical with that of distilled water. Except in table 5, the acid and the sugar plus acid solutions were used

[1] The lemon juice was filtered through cheese cloth.

alternately. An interval of one minute was allowed after the acid or the sugar plus acid solution had caused its reflex before the other solution was used. The same foot was used for all testing during any series of tests. Timing was done by a stop watch except in the work on lemon juice where the second hand of an ordinary watch was used.

When a frog's foot was first exposed to a solution the response was generally slower than in subsequent tests. For this reason the first pair of figures in the protocol of each experiment was discarded and ignored in making averages as they would otherwise distort the general average which was based on a better stabilized condition. Averages for the tables were compiled from protocol that showed fairly comparable figures.

Lemon juice and sugars (table 1). Sucrose and dextrose both very definitely inhibited acid irritation by lemon juice.

TABLE 1

Reflex time of spinal frogs on immersion of one foot in lemon juice and in sucrose plus lemon juice solutions

EXPERIMENT	NUMBER OF PAIRS OF TESTS	TITRATION WITH N/10 NaOH		SUCROSE IN SOLUTION II	AVERAGE REFLEX TIME		PROTECTION
		I Lemon juice solution	II Sucrose + lemon juice solution		I Lemon juice solution	II Sucrose + lemon juice solution	
		cc.	*cc.*	*per cent*	*seconds*	*seconds*	*per cent*
1	2	2.35	2.30	20	20.0	100.0	400
2*	7	4.5	4.5	20	16.3	27.0	66
3	6	4.6	4.7	20	10.7	44.5	316
5	6	9.3	9.5	20	4.2	8.5	104
5	15	8.5	8.5	10	6.8	9.5	42
6	11	9.0	9.0	10	12.0	31.0	156
7†	7	9.6	9.6	10	12.4	21.0	69

* Opposite legs were used for respective solutions.

† In this experiment dextrose was used in place of sucrose.

Citric acid and sucrose (table 2). The results with citric acid were similar to those with lemon juice but somewhat more constant. This was probably largely because citric acid was of a more uniform composition. The results led to the following observations: *a*. "Protection" (or acid inhibition) by sugar was both marked and constant, it being absent in no instance. *b*. The amount of acid remaining the same, a mathematical increase in the sugar content was followed by a somewhat geometrical increase in the degree of "protection." A sucrose content of 5 per cent gave a rather small but a very definite and dependable "protection." Thirty to 40 per cent was almost enough in certain instances to cover the threshold of irritability to the acid. This was true even though the identical

concentration of acid in a solution without sugar (solution I) would on alternate tests cause a very prompt response from the frog preparation. *c.* Citric acid solutions which contained sufficient sucrose to almost or completely cover the acid irritation (and so prevent a reflex to the solution) actually became more irritating when a limited amount of water was

TABLE 2

Reflex time of spinal frogs on immersion of one foot in citric acid and in sucrose plus citric acid solutions

EXPERIMENT	NUMBER OF PAIRS OF TESTS	TITRATION WITH N/10 NaOH		SUCROSE IN SOLUTION II	AVERAGE REFLEX TIME		PROTECTION
		I Citric acid solution	II Sucrose + citric acid solution		I Citric acid solution	II Sucrose + citric acid solution	
		cc.	*cc.*	*per cent*	*seconds*	*seconds*	*per cent*
1	4	16.8	16.7	60	2.5	22.1	794
2	5	10.0	10.0	50	7.1	∞	∞
3	3	9.7	9.7	40	5.0	93.3	1,766
4	7	10.0	10.0	40	7.0	32.8	369
5	6	4.4	4.5	30	6.0	55.7	931
6	5	10.0	10.0	30	7.0	35.0	394
7	5	6.4	6.4	23	5.6	11.2	101
8	4	2.8	2.8	20	10.5	30.7	193
9*	28	4.9	4.9	20	7.6	21.6	184
10	2	9.6	9.5	20	5.5	19.5	245
11	5	10.0	10.0	20	8.0	24.6	205
12	5	5.6	5.6	19	9.0	15.4	70
13	6	4.7	4.6	15	13.2	33.2	151
14	9	9.7	9.7	15	9.5	17.5	80
15	3	2.90	3.00	10	7.3	8.3	14
16	3	5.0	5.0	10	4.3	7.7	77
17	2	9.6	9.6	10	4.5	9.5	111
18	5	10.0	10.0	10	9.2	13.7	45
19	6	2.3	2.3	7	27.7	62.3	125
20	8	9.7	9.7	5	6.6	8.8	32
21	10	10.0	10.0	5	5.8	9.8	56
22	10	10.1	10.0	2	7.5	7.7	2.6

*The figures in this experiment were averaged from the results with four different frogs.

added. The dilution would rapidly decrease the inhibitory power of the highly concentrated sugar, but more slowly affect the hydrogen ion concentration of the acid present in the same solution. Naturally on sufficient dilution the sugar plus acid solution would again become less irritating. *d.* "Protection" was generally greater with frog preparations which showed an inherent sluggishness.

A comparison of various acids—sucrose as the sugar (table 3). Sucrose inhibited all the acids tried although with acetic and hydrochloric acids this inhibition improved slight and was undependable unless over 20 per cent of sucrose was used. Even this did not give any great "protection."

TABLE 3

Reflex time of spinal frogs on immersion of one foot in various acid and sucrose plus acid solutions

EXPERIMENT	NUMBER OF PAIRS OF TESTS	TYPE OF ACID USED	TITRATION WITH N/16 NaOH		SUCROSE IN SOLUTION II	AVERAGE REFLEX TIME		PROTECTION
			I Acid solution	II Sucrose + acid solution		I Acid solution	II Sucrose + acid solution	
			cc.	*cc.*	*per cent*	*seconds*	*seconds*	*per cent*
1	8	Acetic	2.95	3.00	60	10.2	14.5	43
2	7	Acetic	17.4	17.5	40	6.3	6.9	9
3	8	Acetic	1.5	1.45	20	20.8	20.1	0
4	7	Acetic	1.6	1.7	20	25.6	22.1	0
5*	19	Acetic	3.00	3.00	20	14.4	16.8	15
6	12	Tartaric	9.4	9.4	40	2.9	5.8	90
7	7	HCl	0.8	0.8	20	17.4	15.4	0
8	7	HCl	1.5	1.5	20	7.6	9.3	18
9	4	HCl	2.95	3.00	20	4.2	4.7	12
10	8	HCl	1.4	1.4	10	11.4	12.3	7
11	7	Sulphuric	1.0	1.0	40	6.0	86.5	1,344
12	10	Sulphuric			20	8.5	13.7	59
13	8	Oxalic	2.0	2.0	30	3.3	8.0	143
14	11	Oxalic	0.9	0.9	20	5.5	16.4	196
15	8	Lactic	4.8	4.9	30	8.8	53.3	506
16	10	Lactic	5.0	5.0	20	5.4	15.1	181
17	5	Nitric	1.1	1.1	40	11.4	161.0	1,314
18	7	Nitric	2.9	3.0	40	3.3	4.9	46
19	33	Nitric	1.90	1.90	20	8.1	16.9	107

* The figures in this experiment were averaged from the results with four different frogs.

Sugar inhibited tartaric acid fairly well and markedly inhibited sulphuric, oxalic, lactic and nitric acids. Thus the acids may roughly be divided into two groups, the first including acetic and hydrochloric acids where the "protection" by 20 per cent sucrose was slight and undependable, the second including the other acids studied where the "protection" was

quite marked. For convenience the first group is called, "non-protective acids" and the second group, "protective acids."

A comparison of various sugars—citric acid as the irritant. It was questioned whether the phenomenon thus far presented applied to other sugars than sucrose and if so whether the molecular weight of the sugars or their relative sweetness bore any relationship to their protecting powers. Space does not permit the detail of the protocol to be shown in this report. It was necessary that different sugars be used on the same frog preparation. Levulose, dextrose, sucrose and lactose were used in various concentrations. Beakers containing solutions I and II of each sugar were used in turn. Each sugar used in that series was tested before returning to the first sugar again. The process was then repeated. Several frogs were used in various series of tests. The difference in "protection" offered by the various sugars, although not exceedingly great, was definite and quite, although not absolutely, constant. Levulose was most effective, then sucrose, dextrose and lactose. In these tests the sugars "protected" in accord with their relative sweetness. The "protection" was not in any way related to the relative molecular weights.

"Cumulative protection." During the various preceding tests it was observed that sugar "protection" became greater with certain acids as the tests on a frog were repeated. There was apparently a cumulative factor. This principle seemed to apply to acids which were subject to a high sugar "protection" but not to other acids. In the following work citric acid will represent those subject to considerable sugar "protection" and acetic acid will represent the acids subject to only a slight "protection."

It was observed that the cumulative factor was largely lost if the experiment be discontinued for 10 minutes. Consequently a definite portion of the cumulated "protection" must be lost during the one to two minutes extra required for running the acid control each time. To more definitely prove the presence of a cumulative factor the sugar plus acid solution was used alone for a series of tests, then the acid solution was used alone for a similar number of times. The usual technic was otherwise maintained as far as possible. Thus the sugar plus acid tests were made more closely together and no other solution was used between these tests which might distort the results. Tests were also run on the same foot by the old method as a control. Results by the two methods were compared.

Using an approximately N/15 citric acid solution and 20 per cent sucrose, there was on a general average a "protection" by the cumulative method of 430 per cent as contrasted with 274 per cent by the old method. The same foot was used throughout these experiments. The "protection" was very definitely and consistently greater by the cumulative method. It is supposed that this general trend would persist if the leg be immersed in such a sugar plus acid solution indefinitely and that on doing so the cumulative

factor might amount to considerable before its maximum had been reached. Using approximately N/15 citric acid and 5 per cent sucrose, there was an average "protection" by the cumulative method of 96 per cent as contrasted with 51 per cent by the regular method. The cumulative factor was consistently very evident even though a low concentration of sugar was used.

With an acetic acid solution of approximately N/15 strength 60 per cent sucrose gave a "protection" by the cumulative method of 14 per cent as contrasted with 46 per cent by the regular method. Thus, an opposite tendency existed with acetic acid from the tendency with citric acid. Even though a high concentration of sugar was used to emphasize any "protection" that might be present, the cumulative method actually proved consistently and definitely detrimental to a maximum degree of "protection."

Gum acacia with citric and hydrochloric acids. It has been observed that gum acacia will markedly reduce the activity of various medicinal agents. It was questioned whether gum acacia would have the same acid inhibiting characteristics as sugars. About 9 per cent of gum acacia in an N/20 citric acid solution was adequate to cover the threshold of irritability. Response to the acid control (solution I) was in 6 to 8 seconds. Thus only about one-fifth as much of the gum acacia as of sucrose was required to prevent all response to citric acid.

Eight per cent of gum acacia completely covered the threshold of an N/50 hydrochloric acid solution (titration of 2.0 cc.) even though the response to the acid control occurred in 5 to 8 seconds. No concentration of sucrose had been adequate to even very materially inhibit hydrochloric acid. Thus gum acacia was much more effective than sugar in inhibiting hydrochloric acid.

Hydrogen ion concentration. If gum acacia or sugar chemically combine with acids they should alter the hydrogen ion concentration of the acid solutions. Clark's colorimetric hydrogen ion determination was used on the above citric and hydrochloric acid solutions with and without the gum acacia. The gum acacia caused the pH to be very definitely changed to the alkaline side in each case, although the discoloration of the solutions by the gum acacia made any accurate reading impossible.

In contrast, in repeated hydrogen ion determinations in earlier tests the presence of a large proportion of sucrose (60 per cent) in no instance altered the pH of citric or hydrochloric acid solutions. Acid inhibition by sugars is surely not due to a chemical binding of the acids by the sugars.

The "contact response." Solution II (sucrose plus acid) of lemon juice, citric, tartaric, oxalic, lactic, nitric and sulphuric acids had another quality. This quality also applied in tests on citric acid solutions containing levulose, dextrose, lactose or glycerine. When a frog's foot was kept in such

solutions long enough to cause the usual acid reflex it would be set for still another reflex, more violent than the first, on contact with water. The treated leg would start to kick in one to two seconds after immersion in water. There would be about five violent strokes followed by several sluggish ones. Ordinarily the kicking lasted about 4 seconds. The frog would then hang passively. The irritation was evidently very marked, very rapid in its onset and quite brief. The phenomenon seemed to be another characteristic of the "protective" acids.

Hydrochloric acid solutions containing sugar at no time produced this response.

Except for acetic acid, acid solutions without sugar did not lead to this response. Acetic acid with or without sugar resulted in a rather slight and a quite inconstant response on contact of the treated foot with water.

Sucrose was tried alone in solution in concentrations up to 60 per cent. There was no response on immersing the foot in the solution for many (five) minutes, nor on removing it, nor on later immersing it in water.

The use of gum acacia with acids did not cause this response even though gum acacia exerted greater inhibition over the acids than did sugar.

Citric acid solutions were observed containing enough sucrose to entirely prevent any reflex. They still quite readily produced the response on contact with water.

Five per cent sucrose in citric acid solutions set feet for a fairly good response with water. Two per cent of sucrose was not enough for a definite response.

If a foot set for the response be immersed in a sugar solution instead of water there would be no response while there. I generally used roughly the same concentration of sugar as was in the solution II from which the foot was removed. On contact with water after being in the sugar solution for three minutes there would only be the barest suggestion of a response. Apparently the time suspended in the sugar solution permitted most of the citric acid to diffuse out of the epithelium.

Loeb[2] describes a response apparently similar in appearance to that presented above. This he obtained on immersion in water of frog preparations (muscle tissue generally used) previously treated with solutions of salts containing a calcium precipitating ion (as sodium oxalate). He found that once the preparation was set for this response various other substances than water would also initiate the reflex. He stated that the loss of effective tissue calcium caused a marked hypersensitivity such that mere contact with a different medium could cause the reaction. This phenomenon was termed a "contact response."

Loeb did not include work on acid nor on sugar plus acid solutions.

[2] Loeb, J. This Journal, 1901, v, 362.

Consequently, the material presented here does not test Loeb's work nor does it pass evaluation on his explanations although such explanations are of interest. Apparently his theory will not apply in my work, because nitric acid in solution with sugar will set a foot for a good and an apparently typical "contact response" even though calcium nitrate would be very soluble. Doctor Carlson suggested it possible that some electrical factor (couple) might be the cause of the "contact response" as it occurs in

TABLE 4

"Irritation time" (see text) on applying acid and sucrose plus acid solutions to a scarified human lip

EXPERIMENT	NUMBER OF PAIRS OF TESTS	ACID USED	TITRATION WITH N/10 NaOH		SUCROSE IN SOLUTION II	AVERAGE IRRITATION TIME		PROTECTION
			I Acid solution	II Sugar + acid solution		I Acid solution	II Sugar + acid solution	
			cc.	*cc.*	*per cent*	*seconds*	*seconds*	*per cent*
1	9	Citric	3.0	3.0	50	9.2	∞	∞
2*	25	Citric	6.2	6.1	40	1.5	7.8	383
3†	9	Citric	6.2	6.1	40	6.0	18.7	217
4	10	Tartaric	9.4	9.4	40	12.9	32.2	157
5	16	Hydrochloric	3.0	3.0	40	4.5	6.1	36
6	20	Oxalic	4.0	4.0	40	1.7	5.1	198
7	9	Acetic	17.4	17.5	40	11.7	25.1	71
8	10	Acetic	5.0	5.0	40	4.6	14.4	213
9	33	Acetic	24.1	23.9	35	9.3	15.0	74
10	7	Acetic	12.8	12.6	17	14.0	16.4	17

* Solution I also contained 0.6 per cent of sodium chloride.

† Solutions were applied on myself by another person.

my work. Experimental efforts failed to very definitely establish any explanation. A theory is presented later.

Sensitivity as influenced by sugar. Frogs' feet were kept in citric acid and in sugar plus citric acid solutions for various periods of time. They were then gently wiped but no water was applied prior to testing the sensitivity. I tried pinching, scratching, tapping, Faradic current, and the application of the outside of test tubes containing hot or cold water of noted temperatures. Unlike the preceding work none of these tests yielded constant or dependable results even though much work was done. It was tentatively concluded that citric acid reduced the excitability of the sensorium but that sugar inhibited this change. Sugar apparently protected the

nerve endings from the action of the acid but sugar did not cause any unusual sensitivity which would account for the "contact response" nor did it cause any anesthetic effect which would in itself give false evidence of sugar "protection." That much seemed certain.

Tests on the human sensorium (table 4). Tests were made on myself and by myself except in experiment 3 where the solutions were applied by another person. A limited area of the lower lip was rubbed with a piece of moist gauze until weakly acid solutions would fairly readily cause irritation. Solutions were applied with camel's hair brushes. A definite sensation of pain was taken as the end point. After each test the solution was gently sponged off with water and the area was dried with cotton or gauze. Solutions I and II were used alternately at intervals of 30 seconds between each test.

The results rather closely resemble those with frogs. The inhibition of the acid irritation by sucrose was greatest with citric acid, next with oxalic acid, then acetic acid, and least with hydrochloric acid. The difference in the ultimate degree of irritation produced by solutions of citric acid with and without sucrose further emphasized that sugar "protection" was a material factor. With acetic acid marked protection (213 per cent) occurred in one out of five series of tests. In this instance 40 per cent sucrose was used with a weakly acid solution. It seemed that in general the same principles of "protection" applied to the human as to the frog.

I paid close attention to the sensations on washing away the sugar plus acid solution but was not certain of any change analogous to the "contact response" in frogs.

Taste. At least one set of acid and sugar plus acid solutions was tasted with citric, tartaric, oxalic, lactic, nitric, sulphuric, acetic and hydrochloric acids. With all except hydrochloric acid sugar caused a marked and obvious decrease in the acid taste and a pleasing change in the flavor. Contrary to expectation, sugar decreased the acid taste of acetic acid although perhaps not so markedly as with some other acids.

Hydrochloric acid in a concentration fairly well tolerated by the mouth was not noticeably reduced in acid taste by a 40 per cent content of sucrose. Both the acid and the sweet impulses seemed as strong as though they were present in the solution separately. The fact that hydrochloric acid is a mineral acid must mean little in the phenomenon because, in contrast, sulphuric acid was greatly reduced in acid taste by sugar. The sulphuric acid plus sucrose solution had a very pleasing flavor.

The decrease in acid taste by sugar seemed to fairly closely parallel sugar "protection." It seems consequently, that chemical and physiological principles which tend to explain sugar "protection" would also explain the decreased acid taste. It is not necessary nor does it seem advantageous to revert to psychical factors for an explanation of the taste phenomenon.

The influence of sucrose on ordinary chemical reactions of acids (table 5). Half of a dog's tooth divided lengthwise was used in each solution. The halves of teeth used in solutions I and II were in each case made of identical weight before starting the test. The solutions were on an average allowed to act for about 21 hours. The protection derived from 40 per cent sucrose in rather strongly acid solutions varied from 86 to 300 per cent. "Protection" was marked with both citric and hydrochloric acid solutions.

TABLE 5

The relative rate of chemical action of acid and of sugar plus acid solutions

EXPERIMENT	SUBSTANCE TESTED	ACID	SUCROSE	TITRATION WITH N/10 NaOH		AMOUNT OF EACH SOLUTION USED IN TESTS	TIME OF EXPOSURE TO THE SOLUTIONS	ORIGINAL WEIGHT OF SUBSTANCE	WEIGHT LOST		PROTECTION
				I. Acid solution	II. Sucrose + acid solution				I. Acid solution	II. Sucrose + acid solution	
			per cent	*cc.*	*cc.*	*cc.*		*gram*	*gram*	*gram*	*per cent*
1	Dog teeth	HCl	40	3.5	3.5	70	17 hours	0.955	0.041	0.022	86
2	Dog teeth	HCl	25	5.2	5.1	40	27 hours	0.2700	0.0619	0.0554	12
3	Dog teeth	Citric	40	13.7	14.3	40	20 hours	0.110	0.040	0.010	300
4	Dog teeth	Citric	40	15.3	15.3	75	19 hours	0.1103	0.0401	0.0161	149
5	Dog teeth	Citric	40	19.8	19.9	50	17 hours	0.073	0.030	0.0155	161
6*	Dog teeth	Citric	25	17.4	17.4	40	27 hours	0.1145	0.0308	0.0218	41
7	Iron wire, commercial	HCl	30	6.0	6.1	400	15 hours	0.1517	0.0073	0.0032	128
8	Iron wire, C. P.	HCl	40	6.1	6.2	140	(10 days)	0.0487	0.0444	0.0082	441
9	Iron wire, C. P.	Citric	40	15.0	15.0	(140)	6 days	0.0413	0.0081	0.0024	237

* At the end of the experiment the pH of solution I was 2.8 and of solution II was 2.2. Thus, the sugar plus acid solution was very obviously the more acid of the two. The process leading to neutralization of the acid was impaired by the sugar.

A superficial low power examination was made of the sectioned surface of the teeth before and after each test and certain measurements were also made under the microscope. It was found that the corrosion of the enamel was roughly proportional to the gross loss of tooth substance as indicated in the table. Some choose to class sugar as a destroyer of teeth. Sugar certainly gives teeth a very material protection against the corrosion by acids. It is apparent that the increased sugar content of fruits on ripening would certainly inhibit the action on the teeth of the acids present.

Tests on iron wire showed sugar to give an excellent protection against the action of both hydrochloric and citric acids. Hydrochloric acid was inhibited fully as well as citric acid. This is different from the results with animals where decidedly the greater inhibition was demonstrated with citric acid.

Discussion. Sugar inhibits the chemical activity of acids as is proved by the decrease through sucrose in the rate of action of acids on teeth and iron wire. But it seems that sugar does not chemically neutralize nor bind the acids since it does not alter the hydrogen ion concentration of the same in solution. Doctor Carlson suggested that sugar may act by forming a layer of sugar molecules over the surface of objects and thus keep away the acid ions. Any factor active with inanimate objects must also be concerned, when applied to living tissue. Physiological forces may, however, greatly alter the ultimate outcome. It is thought that a protective film of sugar molecules would form over the outside of tissue regardless of the type of acid in the solution. It is thought, further, that with "protective" acids sugar would gradually enter the various cells which come into contact with the solution. Consequently on washing the foot the sugar from the preceding solution would not be entirely lost and on repeated tests a larger amount of sugar would accumulate thus giving the "cumulative" type of protection. The "contact response" would be caused by a swelling and consequent irritation due to a sudden ingress of water into the cells of the epithelium and sensorium, the cells being hypertonic due to the sugar.

It is assumed that with "non-protective" acids for some reason the tissues become impermeable to the sugar. The sugar would still protect by the layer formed over the outside of the tissues. This "protection" as judged by the altered reflex time would be somewhat minimized by irritation from the increased osmotic pressure due to the sugar in the solution. Ordinarily sugar causes little or no obvious irritation in itself but when the barriers against outside substances are in part broken down by the acid then the osmotic factor of the sugar might have a greater effect.

SUMMARY

1. It was found with spinal frog preparations that sucrose greatly reduces the irritation by lemon juice, citric, tartaric, oxalic, lactic, nitric and sulphuric acids. Citric acid was also greatly inhibited by levulose, dextrose and lactose. Hydrochloric and acetic acids were only slightly inhibited by sucrose. The inhibition of acid irritation by sugars is termed a sugar "protection" and it is taken as evidence of an actual protection, not merely a sensory phenomenon.

2. In the work on frogs the inhibition of citric acid by sucrose, levulose, dextrose and lactose was directly proportional to the relative sweetness

of the sugars. It was not related to the relative molecular weights or osmotic power.

3. In the work on frogs the inhibition of citric acid by sucrose showed a very definite cumulative increase in degree on repeated bath in the solutions. With acetic acid the opposite tendency persisted, for on repeated tests the sugar in the solution became less effective in inhibiting the acid.

4. Sucrose has no influence on the hydrogen ion concentration of either citric or hydrochloric acid.

5. When a frog's foot was taken from a sugar plus acid solution of any acid which was greatly inhibited by sugar and was then immersed in water there appeared a brief period of violent kicking, termed a "contact response."

6. In the work on frogs gum acacia gave a "protection" which differed from the "protection" by sugars in that: *a*, less of the gum acacia than of sugar was required for the same inhibition of the acids; *b*, gum acacia very effectually inhibited hydrochloric acid; *c*, gum acacia did not set the foot for any "contact response;" *d*, gum acacia did change the hydrogen ion concentration of the acid solutions toward the alkaline side.

7. In tests on the human lip sucrose greatly decreased the rate and degree of irritation by citric, tartaric and oxalic acids. Sucrose only very slightly decreased the irritation by hydrochloric acid solutions. The tendency was for sucrose to only moderately decrease the irritation by acetic acid solutions. The results with acetic acid were not entirely constant in such tests as were made on man.

8. Citric, tartaric, oxalic, lactic, nitric and sulphuric acids are classed as "protective" acids and they show the following group characteristics when used in tests on the cutaneous sensorium of frogs or when applied in skin tests on man: *a*. They are subject to marked inhibition by sugar. *b*. The "protection" they give becomes greater on repeated application of the solutions (cumulative in nature). *c*. When used with sugar they set the frog's foot for a "contact response." *d*. Sugar greatly decreases the acid taste of such solutions.

The absence of the above characteristics was clearly demonstrated with hydrochloric acid. It is consequently called a "non-protective acid." The tests on frogs show acetic acid to definitely fall into this group, also. There was, however, occasionally a movement suggestive of a "contact response." This was not like the decided "contact response" which would occur with "protective" acids. There was some irregularity in the tests on the human.

9. Solutions of both citric and hydrochloric acids dissolved teeth and iron wire much less readily when there was 25 to 40 per cent of sucrose present. Sugar inhibited the one acid about as readily as the other in this type of test.

This work was done under Dr. A. J. Carlson who offered valuable suggestions and gave a very necessary guidance.

THE EFFECT OF CARBON MONOXID AND OXYGEN AT HIGH PRESSURE ON THE POWER OF ANIMAL TISSUE TO CAUSE THE OXIDATION OF GUAIACUM

ARTHUR L. MEYER

From the Department of Physiological Hygiene, The School of Hygiene and Public Health, The Johns Hopkins University

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Guaiacum has served a useful purpose in many researches. The reader will find an excellent review of the literature bearing on this interesting substance in a bulletin from the Hygienic Laboratory in Washington by Kastle (1). Guaiacum has been responsible, in a large measure, for our knowledge of the existence of three kinds of substances, concerned in biological oxidations: *1*, the oxidases, among them laccase, which render the oxygen of the air sufficiently active to effect the oxidation of easily oxidizable substances directly; *2*, the peroxidases, which activate the oxygen of the peroxids; and *3*, the catalases, which simply cause the decomposition of hydrogen peroxid, without activating the oxygen.

In the present study I have been concerned with three tissues, namely, blood, muscle and brain. Neither of these, in my experience, has shown a capacity of effecting the oxidation of guaiacum directly. If oxidases are present, their action is extremely slow. Although the work of Biedermann (2), Giard (3) and others affords evidence of the existence of laccase in animal tissue, contrary to the prevailing opinion of their time, Kastle points out that such ferments are undoubtedly of much rarer occurrence in animals than in plants.

It has long been known that blood corpuscles dissolved in water give the guaiacum reaction upon the addition of hydrogen peroxid (4). I have repeatedly observed that the success of this phenomenon, in the case of diluted whole blood, depends upon the volumes of blood solution and hydrogen peroxid. A quantity of hydrogen peroxid which enables a given quantity of blood solution to blue guaiacum will fail if twice the quantity of blood solution is used. Again the same volume of hydrogen peroxid may be far more effective if half the original volume of blood solution is taken.

An aqueous suspension of muscle tissue from the hind leg of the mouse appears to be exceptionally efficient in bringing about the guaiacum reaction, but the brain of the mouse is not so active in this respect and the

attempt to demonstrate the oxidation of guaiacum with this tissue may result in failure. Its behavior is quite variable. Brains from different mice are not equally capable of effecting the bluing of guaiacum. A similar difference among mice was brought out a year ago during the course of some direct measurements of the oxygen consumption by brain tissue. However, if the tissue is allowed to stand for three to four hours in a covered dish, its oxidizing action on guaiacum is noticeably greater than it is immediately after removal from the animal. The probable reason for this will be discussed later.

The chief purpose of this communication is to present evidence of the effect of carbon monoxid on the power of blood and muscle to cause the oxidation of guaiacum in the presence of hydrogen peroxid and the effect of oxygen under pressure of retarding the metabolic activity of brain tissue.

EXPERIMENTAL. If a given quantity of blood solution is exposed to carbon monoxid, it loses its power to blue guaiacum in the presence of an amount of hydrogen peroxid which suffices in the case of a quantity of normal blood solution equal to that which was exposed to carbon monoxid. In order to bring about the oxidation more hydrogen peroxid must be added. This experiment was made with human blood, ox-blood and pig's blood.

Experiment 1. Five cubic centimeters of ox-blood were diluted to 25 cc. with distilled water. At 1:50, placed several cubic centimeters of blood solution in a 200 cc. balloon flask. Carbon monoxid (20 cc.) was added to the flask, which was then covered with a black cloth and shaken violently from time to time. At 2:55, placed 0.18 cc. of the tincture of guaiacum and 5 cc. distilled water in each of two test tubes. Normal blood solution (0.5 cc.) was added to the one and carbon monoxid blood solution (0.5 cc.) to the other. Upon the introduction of 0.25 cc. of hydrogen peroxid into each, the normal blood gave rise to the oxidation of guaiacum, whereas the blood exposed to carbon monoxid failed to do so. The further addition of 0.25 cc. of hydrogen peroxid to each simply caused a deeper blue in the tube containing normal blood, but had no effect in the case of carbon monoxid blood. At 3:03, the further addition of 0.1 cc. of hydrogen peroxid to the test tube containing carbon monoxid blood resulted in a greyish brown color. At 3:27, sediment in both about equally blue.

Muscle tissue possesses the property of inducing the guaiacum reaction in the presence of hydrogen peroxid. This might be attributed to the muscle hemoglobin. I have already indicated that this property is exhibited by muscle in a marked degree. It is therefore unlikely that muscle hemoglobin is the sole factor and, if a factor at all, must share its action with another substance having similar properties. Furthermore if muscle tissue is exposed to as much as 10 per cent of carbon monoxid, its oxidizing power is not visibly affected after exposure, showing that there is something else present in muscle which causes the decomposition of hydrogen peroxid and the activation of oxygen.

Experiment 2. Muscle from the hind leg of the mouse. At 12:50, placed 0.318 gram of muscle in bottle 1 and 0.321 gram in bottle 2. Five cubic centimeters of distilled water were added to each bottle. At 1:08, the contents of bottle 2 were placed in a 200 cc. balloon flask and carbon monoxid (20 cc.) was added. The flask was covered with a black cloth and shaken well at intervals. At 3:15, added 0.18 cc. of the tincture of guaiacum to each of two test tubes. The contents of bottle 1 were introduced into one and the contents of bottle 2 into the other test tube. Upon the addition of 0.3 cc. hydrogen peroxid, both tubes showed the same amount of oxidation. The muscle tissue in this experiment was finely divided.

Brain tissue that has been exposed to oxygen under pressure is less efficient in causing the oxidation of guaiacum than brain tissue that has been exposed to atmospheric air for an equal period.

Experiment 3. Mouse brain. Brain divided through the longitudinal fissure into two equal parts. At 11:00, placed both portions in small bottles with 5 cc. of distilled water. The tissue was well macerated. One portion was allowed to remain in contact with atmospheric air, the other was placed in a balloon flask, containing pure oxygen at a pressure of 4 atmospheres. At 2:55, placed 0.18 cc. of the tincture of guaiacum in each of two test tubes and removed the tissue from the high pressure flask. The untreated brain suspension was introduced into one test tube and the oxygen suspension into the other. On the addition of 0.4 cc. of hydrogen peroxid (3:01) there was a rapid appearance of a distinct bright blue color in the case of the untreated tissue, whereas on the addition of an equal quantity of hydrogen peroxid to the tube containing the oxygen suspension (3:02) the bluish tint was greatly delayed and was not as distinct as in the previous case.

Discussion. Schoenbein (5) was of the opinion that the guaiacum reaction of blood was due to the presence of iron, since iron salts were capable of bringing about a similar reaction. The action of carbon monoxid on the behavior of blood toward guaiacum would lead to the same conclusion.

In experiment 1 it will be noticed that the blood solution was kept in the dark during its exposure to carbon monoxid, in order to facilitate the combination of carbon monoxid with hemoglobin. It is possible, therefore, that the absence of light might explain the result. But a control experiment on a sample of blood solution treated in precisely the same way, but without the addition of carbon monoxid to the flask, yielded a positive guaiacum reaction which in one or two instances was even more marked than that yielded by the sample exposed to light.

In spite of the failure of carbon monoxid blood to oxidize guaiacum in experiment 1, there was a marked evolution of gas, indicating that hydrogen peroxid was being decomposed. Senter (6) obtained a catalase from blood, which he called hemase. This substance had no power of inducing the oxidation of guaiacum, even in the presence of hydrogen peroxid. Hemase, then, appears to be unaffected by carbon monoxid, at least in the above concentration.

From the nature of the combination of carbon monoxid and hemoglobin, one would expect some evidence of oxidation of guaiacum in the case of carbon monoxid blood solution, after the lapse of time, owing to dissociation. But an instance of this kind was never observed. A change from a pink to a blue could always be produced, however, by the further addition of hydrogen peroxid. The excess of oxygen thus liberated seemed to cause enough dissociation to enable the characteristic reaction of hemoglobin to appear.

We are justified in concluding from experiment 2 that when muscle is exposed to approximately 10 per cent of carbon monoxid, its behaviour toward guaiacum, after exposure, differs from that of blood. The question whether the positive reaction of muscle is to be explained on the ground that there is nothing in muscle tissue proper that shows an affinity for carbon monoxid comparable to the affinity of hemoglobin or on the ground that an actual combination with some muscle constituent may occur without jeopardizing its function as a peroxidase, is left unanswered. The conduct of the reaction in an atmosphere containing a high percentage of carbon monoxid, as well as a supply of oxygen to meet the oxidative requirements of the tissue, would provide an answer. The result is of interest, however, in suggesting that exposure to such percentages of carbon monoxid as are commonly met with in practice, would give rise to no important after-effect on the peroxidase constituent of striated mammalian muscle.

During another series of experiments, an intensely pink color was produced in the frog's gastrocnemius by an exposure of two to three hours to a concentration of carbon monoxid considerably less than that used in experiment 2. Its ability to do work and recover from fatigue was unimpaired. In this respect it showed no difference from the untreated muscle from the same animal.

Otto Warburg (7) has recently exposed the yeast cell to concentrations of carbon monoxid in the neighborhood of 80 per cent and found the rate at which yeast consumes oxygen is thereby greatly reduced. He attributes this action to a combination of carbon monoxid with the iron, or certain other metals, contained in the yeast cell. It may be of interest, in this connection to note that Schoenbein (8) found that yeast was exceptional in its behavior toward guaiacum in the presence of hydrogen peroxid, in that it decomposes the peroxid, but does not permit the activation of oxygen and the oxidation of guaiacum.

Brain tissue in aqueous suspension, as already indicated, slowly acquires an increased capacity of promoting the oxidation of guaiacum, during a period of contact with air. That this is not wholly due to an increase in the concentration of the extract may be shown by taking one half of the brain, macerating it and spreading it over the inside of a small

bottle, which is then covered. The other half is macerated and placed in a second bottle with 5 cc. of distilled water. Both are allowed to stand for the same length of time. It will then be found that when 5 cc. of distilled water are added to the first bottle, its power to oxidize guaiacum is comparable to that of the second, although its action is somewhat slower.

One is led to believe that brain tissue becomes less energetic as a reducing substance on prolonged exposure to air and that this accounts for the change in its behaviour toward guaiacum. A similar explanation has been offered in the case of laccase. Kastle (9) notes, since Ehrlich (10) found the tissues of the higher animals to exhibit marked reducing properties, that, therefore, the presence of oxidases may be obscured by the powerful affinity of reducing tissues for oxygen.

It may seem difficult to reconcile this explanation with the action of muscle, which also has a high metabolic rate. But it can hardly escape one's observation that the addition of hydrogen peroxid to muscle suspension results in a more marked evolution of gas than its addition to brain suspension. It seems, therefore, that in the case of brain tissue relatively little oxygen is provided for activation and that this just about satisfies the requirement of brain as a reducing substance, whereas, in the case of muscle, more oxygen is activated than the tissue can use, the surplus being diverted to the oxidation of guaiacum.

Attention has previously been called to a peculiarity of blood solution in its action upon guaiacum. It is quite possible that one might fail to demonstrate the guaiacum reaction with blood solution, even though the hydrogen peroxid added might undergo very marked decomposition. Blood contains a potent catalase in hemase, but a peroxidase which appears to be comparatively feeble in its action; so that even in the case of blood, present in excess, the oxidation of guaiacum may be rendered impossible.

If, then, as the result of a diminished rate of oxygen consumption on the part of brain that has remained in contact with air, more oxygen becomes available for the oxidation of guaiacum, one may infer from experiment 3 that exposure of this tissue to oxygen at a pressure of four atmospheres retards its own metabolism, since it now shows a diminished capacity of effecting the guaiacum reaction.

CONCLUSIONS

In conclusion, it may be said that when the hemoglobin of the blood combines with carbon monoxid, its behavior toward guaiacum in the presence of hydrogen peroxid is altered. More hydrogen peroxid is needed to produce the guaiacum reaction than is needed in the case of blood free from carbon monoxid.

On the other hand, carbon monoxid, in a concentration of about 10

per cent, appears to be without any after-effect on the power of muscle tissue to cause the guaiacum reaction.

Oxygen at a pressure of four atmospheres appears to exert a definite retarding influence on the metabolic activity of brain tissue.

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THE EFFECT OF DIFFERENT AMOUNTS OF SEXUAL INDULGENCE IN THE ALBINO RAT

II. Spontaneous Activity[1]

JAMES ROLLIN SLONAKER

From the Department of Physiology, Stanford University, California

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In our former paper on the influence of various amounts of sexual indulgence on growth of the albino rat (Slonaker, 1927) we gave in detail the plan and scope of the experiment. A brief resumé will suffice.

The animals were divided into five groups as follows: A, having no sexual indulgence, consisted of pure virgins and bachelors; B, light breeders, in which but two or three litters were permitted; C, medium breeders, which delivered and nursed as many litters as possible during their life span; D, heavy breeders in which the young were destroyed at birth and the animals rebred as rapidly and as often as possible; E, excessive sexual indulgence without breeding. This group consisted of hysterectomized females and normal males that were constantly together. All these animals were kept in the same room and fed the same well-balanced synthetic food. The only variable factor was the amount of sexual indulgence permitted. Any differences in the results therefore we attributed to this one variable factor.

Some of the animals were kept in revolving cages, to test the effect on spontaneous activity, and the remainder were confined in stationary cages. The number of revolving cages available permitted of but five females from each of the groups A, B, C and D. Group E could not be tested for voluntary activity very successfully because they had to be with the males practically all of the time. It is the results obtained from these twenty females representing five virgins, five light breeders, five medium breeders, and five heavy breeders that will be presented in this paper.

The animals were placed in their respective revolving cages at the average age of 72 days. They spent the remainder of their lives in these cages. They were removed at intervals for a time just sufficient to weight or mate them. The virgins, A, averaged 63 days; the light breeders 83 days; the medium breeders, C, 70 days; and the heavy breeders, D, 70

[1] This research has been conducted with the aid of the Department of Physiology and the Research Fund of Stanford University, and the Committee for Research on Sex Problems of the National Research Council.

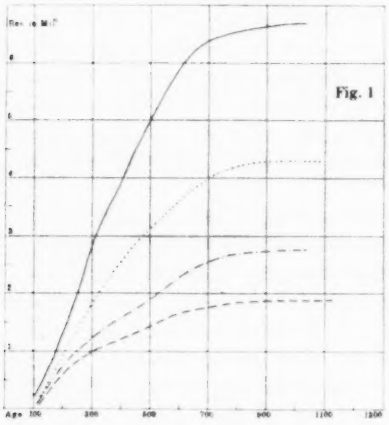
days of age. It has been previously shown (Slonaker, 1924; Wang, 1923) that when females were kept in revolving cages oestrus was always accompanied by a marked increase in spontaneous activity. These rhythmic spurts of activity were absent in sexually immature females, ovariectomized animals and in females which had passed the menopause. Our activity curves indicated that these females had not reached sexual maturity.

When the average total number of revolutions voluntarily turned during the span of life by these different groups of females was considered (see fig. 1) we found that group A, the virgins, surpassed any of the other groups by more than two million revolutions reaching an average total of 6,707,777 revolutions or an equivalent of 5,989 miles. This was over 500 miles greater than the maximum run of 5,447 miles for virgins previously reported (Slonaker, 1912). Group B, medium breeders, averaged the next highest run of 4,313,166 revolutions or 3,851 miles. The heavy breeders, group D, registered 2,763,965 revolutions, an equivalent of 2,468 miles, and the medium breeders, group C, ran an average of 1,899,357, or 1,696 miles.

In accordance with observations previously published (Slonaker, 1925) the explanations for these differences in activity may be easily accounted for. During the period of gestation the voluntary activity of the female drops to about one-third of the normal and during the first ten or fifteen days of lactation it is considerably less. This latter decrease is due to the fact that the female spends almost all the time during the early days of lactation in the nest with the young. There occurs, therefore, with the rearing of each litter a period of thirty or forty days during which the activity is greatly below the normal as exhibited by virgin animals. The light breeders, B, delivered an average of 2.6 litters which would amount to approximately 100 days of reduced activity. The medium breeders, C, had an average of 6.4 litters which would be equivalent to about 220 days. Their total activity would therefore be less than group B. The greater activity of group D, designated heavy breeders, than that of groups C can also easily be explained. We have found that when the young were destroyed at birth normal oestrual rhythm and activity were reëstablished within five to six days after parturition. This group therefore had a period of but about twenty-five days for each litter delivered during which there was reduced activity. Owing to our method of mating these animals there were many cases where successful coitus did not occur for many days after a former delivery. The average number of litters which we were able to secure from this group (D) was 6.6, or only a fraction more than in group C. The total time during which the activity was reduced in this group amounted to approximately 165 days. The order of value of the total average activity of each of these groups is in entire accordance with the

number of days during which they were relatively inactive because of reproduction.

In order to show the distribution of the activity during the life span in these four groups figure 2 has been constructed. These curves represent the total average daily run of each forty-day interval during the total life of each group. By consulting this figure it is seen that the average daily activity increased rapidly and in an almost uniform manner in all groups up to about the age of 150 days. Since the initial increase in activity in young females is associated with the onset of oestruation the slight differences in the curves at the beginning can be explained. The



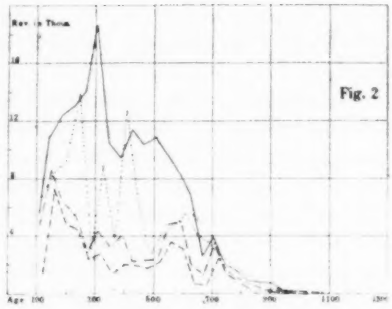


Fig. 1. Average curves of total voluntary activity of rats in revolving cages. Solid line, virgin rats, group A; dotted line, light breeders, group B; dash line, medium breeders, group C; dash and dot line, heavy breeders, group D.

Fig. 2. Average curves of voluntary daily activity of rats in revolving cages. Solid line, virgin rats, group A; dotted line, light breeders, group B; dash line, medium breeders, group C; dash and dot line, heavy breeders, group D.

average age at first oestrus for each of these groups was: A, 74 days; B, 92 days; C, 105 days; and D, 86 days.

The virgins reached an average maximum daily run of 18,705 revolutions at about 300 days of age. Their curve of activity shows what has been previously described (Slonaker, 1926) as long fluctuations in daily activity. It also shows that during the first 600 days this group was very active averaging approximately 11,000 revolutions daily. After 700 days of age the activity of the virgins was very similar to the other groups. This indicates that the amount of sexual activity permitted these rats had little or no effect on the spontaneous activity after the menopause.

The average daily activity of the light breeders, group B, showed four prominent peaks at the ages of about 240, 325, 405 and 610 days respectively. The first was the greatest and reached a little over 13,600 revolutions. The gradual increase up to this high point was disturbed by the birth of the first litter which occurred at the average age of 153 days. The gradual break in the curve was due to the rather wide range in the ages of the mothers at the delivery of the first litter. It ranged from 126 days to 191 days. After the birth of the first litter the animals were allowed to rest for about 80 days. During this time a marked increase in activity occurred. They were then rebred and the birth of the second litter was at the average age of 259 days, with a range of from 243 days to 284 days. This second litter accounts for the sudden drop in activity. The animals were again rested for over a hundred days. Three were then rebred for their third and last litters and delivered young at the ages of 473, 477 and 479 days respectively. This accounts for the sudden drop following the third high point in the curve. We are unable at present to explain the drop which took place preceding this last high peak. It may possibly be one of the long fluctuations in activity. After weaning the third litter there was an increase in spontaneous activity to about that of the virgins. The results of this group indicate that breeding for even a few times reduces the total spontaneous activity of the rat, but if sufficient time intervenes between litters almost normal conditions will be regained.

Groups C and D behaved so nearly alike that their results will be discussed together. The total number of litters thrown by the C group was 32 and the number of young 106; for the D group there were 33 litters and 115 young. The bearing of young was therefore almost the same in each of these groups. The main difference was that group C nursed their young and group D did not. As a result of this the average daily activity of group D surpassed that of the other group during the entire breeding age. The general low activity during the breeding age was due to the reproductive activities. At no time was there a sufficient interval of rest to enable them to reach a normal daily run if they had this power of recovery. After the last litters were born at about the age of 620 days the daily activity increased to about that of the virgins and remained very similar throughout the remainder of the span of life. The results of these two groups indicate that the bearing of young at relatively short intervals greatly lowers the voluntary daily activity of the rat. They also show that the additional nursing of the young still further lessens this activity. The effect produced during the breeding age was apparently not lasting for after the menopause these animals showed practically the same daily activity as that exhibited by the normal virgin rats.

It is interesting to note that the order of average activity of these four groups of animals is in reverse order to that of their growth as reported in

the first of this series of papers (Slonaker, 1927). The virgins (A) reached an average maximum weight of 254 grams and ran 5,989 miles. The light breeders (B) had an average maximum weight of 298 grams and ran 3,851 miles. The heavy breeders (D) reached an average maximum weight of 304 grams and covered a distance of 2,468 miles. The medium breeders (C) had an average maximum weight of 306 grams and ran but 1,696 miles. The differences in weight correlate with the activity and indicate that the food energy which was expended in increased activity on the one hand was used in reproduction and increased body growth in the other case. The bearing of young at an early age which resulted in a lighter maximum weight can now be explained on the basis of increased expenditure of energy in activity during later life.

SUMMARY

When rats were confined in revolving cages in the same environment and subjected to various amounts of sexual indulgence the following results were obtained:

1. During the life span the total average voluntary run for the different groups in the order of accomplishment was A, virgins, 5,989 miles; B, light breeders, 3,851 miles; D, heavy breeders, 2,468 miles; and C, medium breeders, 1,696 miles.

2. The total average voluntary run was in reverse order to the number of litters born.

3. If a sufficient time intervenes between litters average daily activity equal to that of normal virgin controls may be attained.

4. The mere bearing of young did not reduce spontaneous activity so much as bearing the same number of litters and nursing them.

5. The different amounts of sexual indulgence tried did not apparently affect the volitional activity after the menopause. During old age the voluntary activity of each of the groups was very similar and corresponded closely to that of the normal virgin controls.

6 There was a close correlation between activity and growth. The most active group reached the lowest maximum weight and the least active the greatest maximum weight.

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STUDIES ON NERVE METABOLISM

II. Respiration in Oxygen and Nitrogen[1]

R. W. GERARD[2]

From the Kaiser Wilhelm Institute of Biology, Berlin-Dahlem

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The respiratory exchange of isolated peripheral nerve has been studied for a quarter of a century by many workers using a variety of methods, but there has been little quantitative agreement in the results reported. The difference may be in part attributed to variation in the conditions of the experiments and in part to the methods used, some of which have been much less reliable than others. Fenn (1927) has just published much more complete studies on the gas exchange of nerve than have been available, using the lateral line nerve of the dogfish, and he promises similar ones on the frog's sciatic.[3] The results here to be reported, allowing for differences in material and conditions, are in fairly good agreement with those of Fenn, though some differences appear, as will be discussed later.

Recent studies of the heat production of the frog's sciatic nerve give a new interest to data on nerve respiration, as the two may be compared. The absolute value of heat liberated by nerve on tetanization has been determined (Downing, Gerard and Hill, 1926); the relation of heat production to frequency of stimulation and from this the absolute heat produced by a single impulse is known (Gerard, Hill and Zotterman, 1927); and the heat production has been shown to occur in two definite phases (Gerard, 1927a). This at first appeared to indicate a similarity with the lactic acid mechanism of muscle, although the ratio of delayed to initial heat was 9:1 or 6 times as high as the ratio for muscle. A further analysis of heat production in the absence of oxygen (Gerard, 1927b) showed, however, that the two phases could not be separated into anaerobic and aerobic, both

[1] A preliminary notice of this work appeared in "Naturwissenschaften," 1927.

[2] National Research Fellow, U. S. A.

[3] This work has now appeared, Journ. Gen. Physiol., 1927, x, 767, and contributes further important data. Particularly, the oxygen penetration has been shown adequate in the case of frog nerves, and the results therefore free of the objections possible in the case of the thicker dog-fish nerves. The resting oxygen consumption per gram and hour is 74 cmm., and the increase on continued stimulation at 280 shocks per second about 22 cmm., at 22°C. These values should be used in place of those given in the text in the discussion.

phases falling slowly and equally as conduction gradually failed in nitrogen. Such a result indicated that nerve obtains energy for activity, in the absence of external oxygen from a store of oxidizing substances rather than by anaerobic breakdowns.

It appeared highly desirable, therefore, to make comparable studies on the chemical events of rest and activity of nerve, both to obtain absolute values and to test the conclusions reached from the heat experiments. This work has been carried out in Prof. Otto Meyerhof's laboratory, which, because of the similar investigations of muscle carried out there, was especially suited to it; and for the use of these facilities as well as for his interest and advice I am greatly indebted to him. The experimental conditions, where possible, were made the same as for the heat investigations, and the results obtained have been in very good agreement with those on heat.

METHOD. a. *Manometers.* The manometric methods of Warburg and his collaborators (1926) have been used throughout for the measurement of oxygen taken up or carbon dioxide produced; various types of manometers serving for different phases of the work.[4]

The experiments on oxygen consumption of resting nerve were performed in simple chambers of 3 to 4 cc. volume with an inset for alkali. They were usually used with 0.1 cc. $\frac{N}{1}$KOH in the inset and nerves and Ringer solution (with special additions as desired) to a volume of 0.5 cc. in the remainder. Under such conditions the constants for the six chambers were all about 0.4, so that 2.5 mm. displacement of the fluid column represented 1 cmm. change in gas volume. Manometer readings are easily made to 0.5 mm. and the sensitivity was, therefore, 0.2 cmm. Actual volume changes observed in prolonged experiments (15 to 20 hours) were often more than 100 times this quantity and may therefore be considered accurate within a very few per cent. Equilibrium of temperature and gas distribution was obtained by continued motion in a water thermostat, and temperature or atmospheric pressure changes were cancelled out by appropriate controls. In some cases the moist nerves were used without Ringer solution.

For obtaining the carbon dioxide production of resting nerve several methods were employed, all alike depending on the determination of the R.Q. rather than direct measurement of the carbon dioxide alone. The "box" chambers for simultaneous measurement of carbon dioxide and

[4] These methods apply only to systems in which one or two gases change in quantity. In nerve, small amounts of NH_3 seem to be evolved besides the O_2-CO_2 exchange; but fortunately this does not disturb the manometric readings, as it is extremely soluble in water, and under the conditions of these experiments would give less than 1 per cent as great a reading as an equal quantity of O_2 or CO_2.

oxygen, using different quantities of fluid, (Warburg, 1926) were fairly satisfactory. The most positive method, however, involved the estimation of total carbon dioxide, the carbon dioxide combined in the nerve being freed by addition of strong acid $\left(\frac{N}{1}HCL\right)$. In two vessels, with side bulbs containing the strong acid, opposite nerves from the same frog were placed. One was then tipped, allowing the acid to reach the nerves, as soon as the system was in equilibrium to obtain preformed carbon dioxide, the other tipped after the desired time to give total bound carbon dioxide. The carbon dioxide freed during this time was obtained from the displacement of the manometer columns after the oxygen consumed had been allowed for. The oxygen consumption was measured in a third chamber on nerves from similar frogs, having alkali instead of acid in the side bulb. The sensitivity of these vessels was about two-thirds as great as that of the simple ones.

In one group of experiments it was necessary to replace nitrogen with oxygen and secure equilibrium as soon as possible. To this end the simple chambers were fitted with a stopper containing a capillary and stop-cock, instead of the usual solid one. With this as an inlet and the usual passage to the manometer as an outlet, the cock here being open, gas could be passed through the chamber without removing it from the thermostat or touching the chamber itself.

The experiments on stimulated nerve were all performed with a differential manometer. Caproic acid was used as the measuring fluid (Warburg, 1926), the capillary was fairly small, radius 0.25 mm., and the chambers identical, each with a volume of about 3 cc. Each chamber was supplied with an inset for alkali and two pairs of platinum electrodes fused through the glass, one for stimulating and the other to lead off the action current (fig. 1). The electrodes were insulated except at their tips with paraffin, which was also used to bring the two chambers to exactly equal volume. The constants for the manometer were calculated by Warburg's formula (1926) and also measured directly by forcing a known volume of gas into one chamber, as suggested by Hoffmann (1913). The results agreed within 5 per cent and showed that a difference of 1 mm. in the columns represented almost exactly 0.5 cmm. difference in volume on the two sides. This constant is theoretically independent of the total pressure in both chambers and was found by test to be so over the range met in these experiments. The fluid levels were read with a lens arranged to slide on the manometer limbs, and parallax errors were avoided, so that readings accurate to 0.1 mm. were easily obtained. Duplicate readings were made throughout. The instrument was sensitive, therefore, to at least 0.05 cmm., though occasionally temperature variations of the part of the manometer exposed to air caused fluctuations several times this great. Later, smaller cham-

bers, volume 1.5 cc., were used, and the sensitivity made 1.5 times as great.

Opposite nerves of several frogs (usually four) were put in each chamber. The central ends were passed between and bent back over the stimulating electrodes, the crushed peripheral end and uninjured side lay on the two leading-off electrodes. The nerves were arranged parallel and touching each other laterally and the floor of the chamber below, and were either just moist with Ringer or with 0.1 cc. extra; 0.2 cc. $\frac{N}{1}$ KOH was used in the insets when oxygen measurements were to be made, none if carbon dioxide was being measured. The resting metabolism of the two sets of nerves was balanced out in this way and the excess resulting from stimulating one side could be measured directly, deflections of 5 mm. or more being obtained on stimulation for an hour.

b. *Stimulation.* A Harvard induction coil giving 180 make and 180 break shocks a second, the same coil employed in the heat experiments, was used throughout. The secondary coil was removed to the end of the slide (12 cm.) and tilted at 40 degrees from horizontal (in the same cases it was used horizontal) in which position it delivers, with two volts in the primary, slightly supermaximal stimuli, as tested by the action currents. Stimulation was usually continued one to several hours, either continuously or intermittently. For the intermittent stimulation there was inserted in the primary circuit a clock arranged to make contact and so close the primary circuit regularly for 22 seconds every 4 minutes.

In a few experiments on the effect of frequency of stimulation on oxygen consumption, a calibrated spring dipping in mercury and vibrating under the control of an electro-magnet (Bernstein interrupter) was used. Frequencies of 50, 100 and 280 were employed. The make and break shocks were not exactly evenly spaced, but were near enough for a rough estimate of frequency effect. The disagreeable sparking at the mercury contact (requiring an alcohol drip to keep it clean and giving irregular shocks) was eliminated by connecting a 10 mfd. condenser across it.

c. *Action current.* This was led off either; 1) as previously described from polarizable electrodes through a 10 mfd. condenser to a high resistance moving coil Siemans-Halske galvanometer, (sensitivity at 1 meter; 1 mm. $= 3 \times 10^{-10}$ amp.) which responded with a ballistic throw, or 2) from Ag, AgCl electrodes and balanced in the usual way. The action current was used as an independent means of following the condition of the nerve, especially in nitrogen experiments.

d. *Gases.* Oxygen with or without 5 per cent carbon dioxide was used directly from a cylinder. Nitrogen containing 5 per cent carbon dioxide was purified of oxygen by passing over glowing copper filings. In all other cases nitrogen was obtained entirely oxygen free by forcing the gas

in minute bubbles through a porcelain filter into a meter-long column of hydrosulfite, as described by Kautsky and Thiele (1926). All the connections were glass or lead pipe except the final lead from the wash bottle with Ringer solution to the manometer, which was rubber. The manometer was flushed with a good current of this nitrogen for several minutes, with frequent shaking, the air was forced out of the capillary by raising the fluid level, and the stop cocks were finally closed under a slight positive pressure. When oxygen was to be admitted to a chamber containing only nitrogen, the latter was displaced with air or oxygen. The simplest arrangement in case of air was to connect a rubber bulb of about 15 cc. capacity to the capillary stopper, open both cocks, rapidly squeeze the bulb, and shut the cocks again. The whole process took only a few seconds and the manometer remained in the thermostat, so that a zero reading could be made under fairly constant conditions at once on closing the cocks. With this method there was always a considerable decrease of volume in the first five minutes after the air was blown through, and although, with care, it could be rendered quite constant, it tended to obscure the physiological effects. To eliminate it as far as possible, oxygen was led through a long lead coil and a wash bottle containing some weak alkali, the whole submerged in the thermostat, and finally by branching glass and rubber connections directly to each manometer. A constant head of pressure was maintained and the manometers flushed with oxygen by opening the stopcocks, as with air. The entering gas was therefore free from carbon dioxide and of the correct temperature, and to further facilitate equilibrium the nerves in the manometer were not lying in Ringer solution but were suspended in the gas space. The incidental volume changes following the exchange of gases were thus practically eliminated.

In some experiments, oxygen of varying low concentrations was used. These were obtained by diluting air with appropriate amounts of oxygen-free nitrogen, the mixing being done in a 3 liter bottle by displacing water.

e. *Nerves.* Rana esculenta and Rana temporaria were used. Various batches in different conditions of nourishment, temperature, size, etc., gave varying quantitative results though the general agreement was good.

Nerves were dissected rapidly and carefully by a routine technique. As a rule during the entire preparation no twitch of the lower leg muscles was produced until the nerve was cut at its ends for removal. No threads were tied to the ends in these experiments, the nerves being handled with fine glass needles and hooks. The nerves were obtained practically without blood contamination by allowing bleeding through cuts in the viscera before dissection. The nerves to the spinal column were easily freed from the vascular membrane in temporaria and with somewhat more difficulty in esculenta, handling being reduced to a minimum throughout. The left nerve was always dissected first. Two to five nerves were used in each

chamber in all experiments, and when two chambers contained equivalent nerves these were placed alternately, so that the first chamber received the left nerve of the first frog, the right of the second, left of the third, etc. This was done to eliminate any constant difference in dissection technique on the two sides and in the time the nerve remained in the frog after killing and in Ringer after dissection. The time in the frog for both nerves was never more than 5 minutes after killing, but the nerves remained in Ringer $\frac{1}{4}$ to 1 hour, occasionally even longer, before the experiment was under way. In several experiments the frogs were cooled in ice before dissection and the nerves kept in iced Ringer till used; this made no difference in the results.

When all the nerves were prepared for one series they were removed from the Ringer, blotted on filter-paper, weighed on a Hartmann-Braun torsion balance, and transferred to the manometer chamber, where they either were freely suspended in Ringer or hung from the walls in the gas space. The manometer was then filled with an appropriate gas, transferred to the thermostat and allowed to reach equilibrium for 20 to 30 minutes before readings were begun. A single esculenta nerve weighed 25 to 40 mgm., a temporaria nerve 20 to 30 mgm.

The experiments were often continued 15 to 20 hours and no special precautions were taken against bacteria. That these did not affect the results is shown by the following: no evidence of growth was ever observed; chambers containing Ringer or Ringer and dead nerve (killed by boiling, acid, etc.) run for similar or longer times gave no signs of gas exchange; the observed metabolism with live nerves remained quite constant over this period or showed a slight fall—never increased; and the temperature used, 14°C., was unfavorable to most bacteria.

Frog Ringer without $NaHCO_3$ was used regularly. In some of the experiments on carbon dioxide production, when gases containing 5 per cent carbon dioxide were used and no alkali was present in the inset, 20 per cent by volume of a 1.3 per cent $NaHCO_3$ solution was added to the Ringer.

f. *Temperature.* With the exception of a few specified observations, all the experiments were carried out at 14°–7°C.

Results. *Part I. The Gaseous Exchange of Resting and Active Nerve.* a. *The oxygen consumption of resting nerve.* Over 60 experiments on 180 nerves gave quite consistent results. For winter esculenta nerves the extreme values were 11 to 21 cmm. oxygen consumed per hour per gram of nerve with an average of 16.0. For temporaria the extremes were 17 to 27, with an average of 23.0 cmm. per gram per hour. Spring frogs, after the mating season, gave considerably higher values, 21 for esculenta and 28 for temporaria.[5]

[5] The rather large differences observed in resting O_2 consumption, which seems to apply also to the excess of activity, are due only on part to differences in temperature

Control experiments showed that the order of preparation, temperature (room or ice) of preparation, time taken and degree of handling during preparation, within reasonable limits, and whether kept in Ringer or gas in the manometer, were immaterial. The opposite nerves of the same frog showed an average difference of about 10 per cent in their oxygen consumption.

A slight but definite correlation exists between the size of the frog and nerve and the resting metabolism. In esculenta, for example, the average oxygen consumption per gram and hour for different sized nerves was: for 25 to 29 mgm. nerves, 19.0; for 30 to 35 mgm. nerves, 16.4; and for 36 to 40 mgm. nerves, 14.5. The higher metabolism of smaller nerves may very easily be attributed to their coming from younger frogs. Fenn (1927) has just reported a similar correlation between the thickness of nerve and the oxygen consumption per unit weight, in the case of the lateral line nerve of the dogfish. Some of his nerves had a diameter of almost 5 mm., and as he realized, the inner fibers were probably asphyxiated even with the whole nerve in air. He attributed the greater oxygen consumption of smaller nerves to the more complete participation of all the fibers, being unable to determine whether any true difference in metabolic level existed. In these experiments the oxygen diffusion factor is absent (see below) and the effect, therefore, a purely biological one.

The various parts of a mixed nerve like the sciatic have the same metabolism, the oxygen consumption of the upper and lower halves of the same nerves being equal. This does not eliminate the possibility of gradients in individual fibers. (Child, 1924.)

The oxygen consumption of isolated resting nerve remains quite constant over at least 20 hours. Sometimes the values for the first hour or even two are higher than the later average, by as much as 100 per cent; more often the rate is almost or quite constant from the start of observation ($\frac{1}{2}$ hour after dissection). This initial excess, similar to the initial carbon dioxide excess reported by Parker (1925), was especially noticeable in starved frogs, in which the rate also slowly fell during the later periods, but it was also observed in normal frogs especially when the handling had been too rough.

One constant source of injury and therefore of possible error is the cut ends of the isolated nerve. Cutting a nerve into short bits (2 to 3 mm. long) markedly increases the oxygen consumption for several hours, often by more than 100 per cent; the effect is generally entirely over in 4 or 5 hours and a constant rate of the usual value established. The isolation of the nerve may therefore easily explain the early excessive values, but con-

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or state of nourishment. There seems to be a true burst of metabolism in the entire animal about the time of mating.

versely it may be fairly assumed that the later values give a true measure for the metabolism of the resting nerve.

As a more extreme case of cutting up the nerves, several experiments were made on nerves thoroughly crushed in a mortar. These also showed a great excess oxygen consumption at first (as much as 200 per cent), which rapidly fell to $\frac{1}{2}$ to $\frac{1}{4}$ of the normal value. A quite considerable oxygen consumption continued throughout the twenty hours; an observation in harmony with those of Thunberg (1923) on the oxidizing activity of chopped nerves.

Nerves killed by immersion in liquid chloroform for several minutes showed a very similar oxygen consumption—excessively high at first and continuing at a low value (about 10 per cent of normal) for many hours. (The effect was not an artifact due to absorption of chloroform vapor by the alkali in the manometer.) In contrast to nerves killed by crushing or chloroform, those killed by boiling, acid, or alkali gave no oxygen consumption at once or later. The two former agents apparently spare parts of the oxidizing mechanism. Potassium cyanide, even in $\frac{N}{100}$ concentration, did not abolish entirely the resting oxygen consumption.

When glucose was added to the Ringer solution containing normal nerves to a 0.5 per cent concentration, the oxygen consumption was definitely decreased (15 to 20 per cent). A concentration of 0.1 per cent glucose had no effect. Sodium lactate, 0.1 per cent, also had no effect, though stronger or unbuffered solutions caused some decrease.

In several experiments the same or equivalent nerves were kept at 14.6°–20.3° and 28.2°C. and the oxygen consumption measured. The rates were in the ratio of 1:1.65:2.65, which give a very nearly linear relationship and correspond to $Q_{10} = 2.2$ (15–25°C.).

b. *The carbon dioxide production of resting nerve.* The production of carbon dioxide is secondary in importance to the oxygen consumption as a measure of activity, its main value is, through the R.Q., in permitting an estimate of the type of fuel being burned. The measurement of the true metabolic production of carbon dioxide is also complicated by the possible retention of it in the tissue, on the one hand, and by the possible driving out of previously bound carbon dioxide by stronger acids, on the other.

The R.Q. of esculenta and temporaria nerves was determined directly by the methods mentioned. The most reliable results were obtained by the driving out of all carbon dioxide with strong acid. Six such experiments gave values for the R.Q. between 0.64 and 0.90, with an average of 0.75. The protocol of a typical experiment is given in table 1. Six experiments using the "box" chambers gave values between 0.51 and 0.97, average 0.80. The R.Q. of resting nerve may therefore be taken as near 0.77, and the carbon dioxide production, in cmm. per gram nerve per hour, for winter esculenta as 12.0, for temporaria 16.0. The values for carbon

dioxide cannot be considered so exact as those for oxygen since they involve several variables, but the error is certainly only one or two in the second digit. It is worth noting that the acid method gave a somewhat lower R.Q. than the simple manometric one, which seems to show that no error was introduced by carbon dioxide retention in the latter. The rather low R.Q. obtained is probably due to the use of starving winter frogs in these experiments; well-nourished spring ones might be expected to show a higher ratio. Fenn (1927) reports a very similar R.Q., 0.83, for dogfish nerves.

TABLE 1

Experiment 2/21

(R.Q. in side bulbs)

	No. 1. $K_{CO_2} = 0.59$	No. 2. $K_{CO_2} = 0.59$ $K_{O_2} = 0.49$	No. 3. $K_{O_2} = 0.55$
	5 temporaria sciatics, weight = 134 mgm., in 0.8 cc. plain Ringer, 0.2 cc. N/1 HCl in side bulb	Like No. 1, the 5 equivalent nerves, weight = 133 mgm.	5 temporaria nerves, weight = 131 mgm., in 0.8 cc. plain Ringer, 0.2 cc. N/1 KOH in side bulb
0 hour	Tipped $h_{CO_2} = +14.9$ preformed		
17.5 hours		H = −31.1 Tipped $h_{CO_2} = +17.2$ bound	h = −72.2
		If $Q_{O_2} = -17.4$, $h_{O_2} = -83.0$ and $h_{CO_2} = +51.9$ $h_{CO_2\ total} = +69.1$ Total CO_2 = 306 cmm. per gram	$Q_{O_2} = -17.4$
	Preformed CO_2 = 66 cmm. per gram	Formed CO_2 in 17.5 hours = 240 cmm. per gram $Q_{CO_2} = 13.7$ *R.Q. = 0.79*	

c. *The extra oxygen and carbon dioxide exchange during activity.* The extra oxygen consumed by nerve as a result of activity depends on the type of stimulation as well as on the various factors that control the resting consumption. The frequency of stimulation, in shocks per second, and the length of stimulation if continuous or the alternation of stimulation and rest if intermittent are fundamental in determining the amount of activity induced in the nerve. Twenty experiments were performed as described, using a differential manometer to balance out resting metabolism, and stimulating one side with 280 shocks a second, either continu-

ously for one or more hours, or for 22 seconds every 4 minutes for several hours. The results were quite clear cut and consistent. For the continued stimulation the excess oxygen per gram per hour of stimulation averaged 18.0 cmm., the individual variation being 11–27. Lower values were given by esculenta than by temporaria, and spring frogs gave higher ones than winter frogs. For intermittent stimulation the excess of oxygen averaged 61 cmm. per gram and hour of stimulation, the variation being 48 to 91 (table 2).

The passage of the stimulating current was not responsible for the decrease in volume, for: 1, when the air or oxygen in the chamber was re-

TABLE 2

DATE	NERVES USED	EXTRA OXYGEN CONSUMED (CMM. PER GRAM PER HOUR STIMULATION)	
		Continued stimulation	Intermittent stimulation
1/21*	4 Esculenta—175 mgm.	15.5	
	4 Esculenta—180 mgm.	15.0	
1/28	4 Esculenta—150 mgm.	21.0	
	4 Esculenta—155 mgm.		54.5
2/5	4 Esculenta—145 mgm.	11	48
	4 Esculenta—150 mgm.		50
2/8	4 Esculenta—220 mgm.		48
	4 Esculenta—220 mgm.		52
2/22	4 Temporaria—90 mgm.	19	
3/3	4 Temporaria—65 mgm.	27	
3/11	4 Temporaria—80 mgm.	19	72
3/24	4 Esculenta—150 mgm.		68
	4 Esculenta—150 mgm.		80
3/28	4 Esculenta— 90 mgm.	15.5	
3/31	4 Esculenta— 80 mgm.		91
5/7	4 Esculenta—110 mgm.	17.5	
5/19	4 Esculenta—180 mgm.	14	50

* Two separate experiments on the same date were always performed on equivalent nerves from the same frogs.

placed by nitrogen the decrease disappeared, and 2, when the nerves were arranged on the electrodes as usual but cut just below them, so that the nerve impulses set up were limited to the small bits lying on the electrodes, there was again no decrease in volume. As a matter of fact, there was during the first five or ten minutes after stimulation began, especially when the coil was horizontal and shocks were supermaximal, an increase of volume which was later reversed when stimulation ended.

By making observations at 15 minute intervals or less, it was possible to follow the course of the extra oxygen consumption. An entirely typical experiment is shown in figure 2, and it will be observed that the extra oxy-

gen consumption does not jump suddenly to full value at the start nor disappear at the close of stimulation, but shows a lag of half an hour in reaching the maximum and perhaps even longer in falling again. That this lag is not entirely an artifact is shown by the following. The effect of the stimulus itself, mentioned above, would of course give an apparent lag by subtracting during the first quarter hour of stimulation and adding to the first of rest; but the quantity is too small to account for much of the observed lag, and the effect is practically limited to the first 5 minutes and can so be subtracted. A slow arrival at equilibrium in the system would also contribute to such a lag as observed. A considerable increase in the speed of shaking the manometer had no effect on the lag. It has been calculated (Gerard, 1927b) that a nerve of these dimensions exposed to oxygen will become practically saturated in about two minutes, and these nerves were in contact with the gas through only a thin film of Ringer solution. A delay of diffusion out of the nerve of carbon dioxide would, if anything, bring about the reverse effect, that is, an apparently greater decrease in volume; but a delay of absorption of the liberated carbon dioxide by the alkali is to be considered. Control experiments have shown that the carbon dioxide admitted to the chamber is entirely absorbed within five minutes. Also when no alkali is present and the nerves stimulated there should be, if carbon dioxide were freed from the nerve and accumulated in the gas space more rapidly than oxygen was taken up, a rise in pressure at the start and a fall at the close. This effect does not appear. As a final control on the whole system, it was desirable to produce in the manometer chamber a sudden known increase in oxygen consumption and carbon dioxide liberation. This was done by adding (by tipping the manometer) a drop of 1 per cent glucose solution in 1.5 per cent KH_2PO_4 solution to a yeast (Torula) suspension in the phosphate solution; which has been shown by Meyerhof (1925) to promptly increase its metabolism about 10 times. The results are shown in figure 3, and it is clear that the system has practically reached its new equilibrium within 10 minutes. It may be concluded, therefore, that the lag observed on stimulation of nerve is physiological. Figure 4 shows semi-diagrammatically the probable course of oxygen consumption during rest and stimulation. Fenn (1927) observed a similar lag in dogfish nerves, but due to the thickness of his preparations, he was forced to attribute it largely to delayed oxygen diffusion. He does suggest, however, in addition a true "recovery" oxygen consumption (p. 338).

In two experiments of continued stimulation for three hours the extra oxygen consumed during the last hour was more than $\frac{2}{3}$ as much as during the first.

Two experiments with the Bernstein interrupter gave, for continued stimulation at 280 shocks per second, the same oxygen consumption as

with the coil. Stimulation at 100 per second gave as much as 280, 50 per second about $\frac{2}{3}$ as much.

In one experiment the values of extra oxygen consumption on stimulation at 14°C. and 28°C. were determined. The Q_{10}(15° to 25°C). so obtained was 2.1, the same as that for the resting metabolism.

The R.Q. of the excess metabolism of activity is determined by omitting alkali from both chambers and stimulating one. The resting exchange is cancelled out and any change of volume on the stimulated side is due to oxygen used (−), plus carbon dioxide, freed (+). If the R.Q. is 1.0 there is no change on stimulating one side (with the small amount of fluid, the constants for oxygen and carbon dioxide are almost equal); if there is a change of volume the R.Q. can be obtained at once if the oxygen consumption is known. This can be determined on the same nerves later or be assumed from the known value for similar nerves. Five experiments gave R.Q.'s of 0.85, 0.95, 0.99, 1.00 and 1.00; average 0.97. In this connection the question arises whether it is justified to assume that the resting metabolism, with a lower R.Q., continues unchanged and the excess, with its

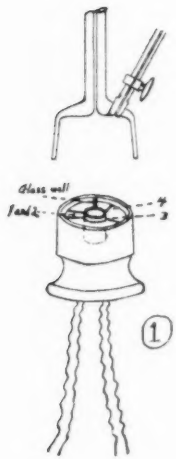
Fig. 1. Nerve chamber of differential manometer. The central insert is for alkali when desired, the nerves being arranged in concentric rings about it and in contact with the electrodes. Electrodes 1 and 2 for stimulation, 3 and 4 for leading off the action current.

Fig. 2. Rate of change of fluid levels in the two limbs of the differential manometer with respect to each other, showing increased oxygen consumption of nerve as a result of continuous stimulation. The difference in height of the measuring fluid in the two limbs (right minus left) is measured at regular intervals (in this case 15 minutes) and the change in this difference is plotted *vertically*. *Plus* represents a relative decrease in volume in the left chamber, *minus* a decrease in the right. *Horizontally*, time. Four equivalent nerves in each chamber, weight circa 100 mgm.; alkali present in both chambers. Stimulation indicated by heavy lines.

Fig. 3. Control on speed of response of manometer to change in rate of oxygen consumption. Same coördinates as figure 2. Yeast suspension in each chamber. At time marked by ↓, glucose added to yeast in left chamber by tipping manometer.

Fig. 4. Course of oxygen consumption during rest and activity; semi-diagrammatic. *Vertically*, oxygen consumption in percentage of the maximum consumption during continued stimulation. *Horizontally*, time in minutes.

Fig. 5. Oxygen consumption by nerves after a period in nitrogen. *Vertically*, manometer reading (corrected). *Horizontally*, time after admission of air. The dotted portion of *C* represents the overnight rate. *A*, after 20 minutes in nitrogen—first period; *B*, after 2 hours in nitrogen—second period; *C*, after 20 minutes in nitrogen—third period; *D*, after 2 hours in nitrogen (or air), the nerves having first been killed by boiling. The dots on *B* and *D* indicate the actual readings. The straight lines continued from the curves to the Y axis indicate the resting oxygen consumption and permit the calculation of the excess oxygen consumed. In this case, the decrease in volume after the nerve was kept 2 hours in nitrogen was 5 mm. greater than when kept 20 minutes. The manometer constant is 0.40 and the vessel contained three Temporaria nerves, weight 82 mgm., so the excess oxygen taken up was 23 cmm. per gram nerve.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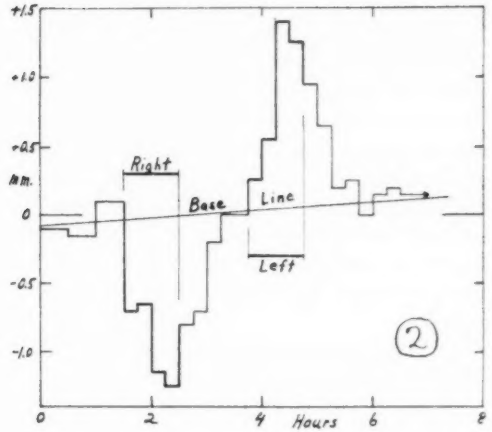
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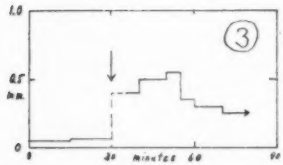
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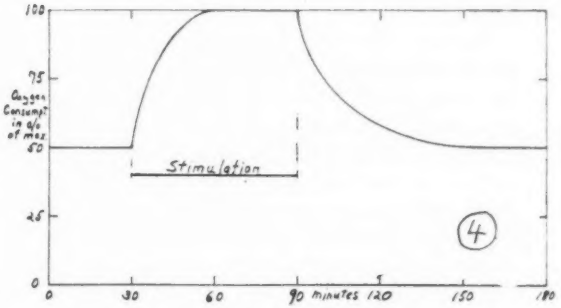
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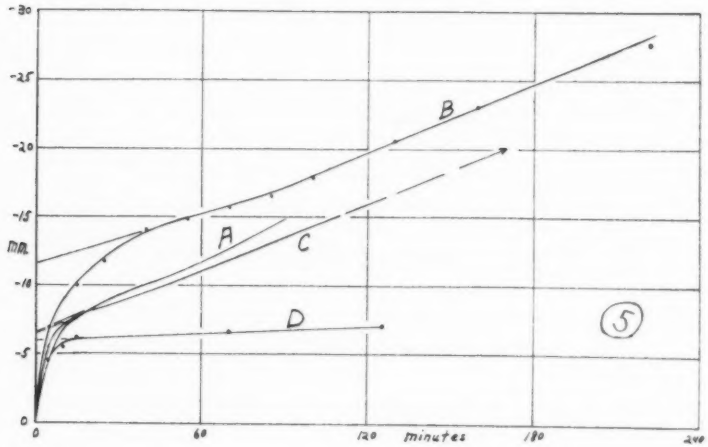
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R.Q. of 1.00, is simply added to it during stimulation; for the possibility exists that during activity the resting metabolism is also modified. That the former situation prevails is shown by an experiment in which nerves were put only in one side of the differential manometer, no alkali being present. The resting metabolism, with an R.Q. about 0.8, caused a small steady decrease in volume, which was absolutely unmodified during or after stimulation. This shows again an R.Q. of excess metabolism of unity and suggests a fairly sharp separation of resting and active metabolism; a separation which may depend on different processes in each case, or may mean simply that activity is limited to a small fragment of the structures of the total tissue, so that the larger part continues an undisturbed resting metabolism.

Fenn (1927) has found a much lower R.Q. on stimulation, which he attributes on good grounds to the retention of carbon dioxide. It is not entirely clear why such a retention occurred in his nerves, as none was met in these experiments. The different conditions and especially the larger size of his nerves may explain the difference.

Part II. The Rôle of Oxygen in Nerve Metabolism. a. *The oxygen supply of isolated nerve.* The question of oxygen diffusion into isolated nerve has been considered for the case of nerves lying parallel in lateral contact so as to give essentially a sheet of tissue (Gerard, 1927b). For esculenta nerves of diameter 0.059 cm. arranged in this fashion and exposed to gas only on one face, it was calculated from Warburg's (1926) formula, Krogh's (1919) diffusion constant, and the known oxygen consumption, that an oxygen concentration of 5 per cent in the gas would suffice for the resting metabolism and one of 15 per cent for the metabolism during activity. In the case of separate nerves suspended freely in liquid or air and able to take up oxygen from the entire circumference, the formula for a tissue slice is not applicable, and Doctor Szilard[6] has kindly helped me develop a similar equation for a long cylinder. The argument is briefly as follows. For a tissue cylinder of radius r and unit length, using a quantity of oxygen A per unit weight and time, the oxygen required in unit time is $A\pi r^2$. The oxygen that will diffuse into the tissue in the same time is given by the surface times the diffusion coefficient for oxygen, D, times the oxygen gradient in the tissue, or $2\pi rD\frac{dC}{dr}$ where C is oxygen concentration at radius r. In a condition of equilibrium the oxygen entering the cylinder must equal that used, so that

$$A\pi r^2 = 2\pi rD\frac{dC}{dr}$$

or

$$\frac{dC}{dr} = \frac{A\,r}{D\,2}$$

[6] Assistant in the Institute of Theoretical Physics, University of Berlin.

Integrating,

$$C = \frac{A\ r^2}{D\ 4} + K$$

At $r = 0$, $C = K$; and for the limiting case, where the tissue all has oxygen available but no excess is present, $C = 0$ when $r = 0$, and therefore $K = 0$. The concentration of oxygen, C_0 needed at the surface of a given tissue cylinder of radius r_0 in order to just permit all parts to receive oxygen is therefore given by the equation

$$C_0 = \frac{A\ r_0^2}{D\ 4}$$

It appears then that a cylinder of tissue takes up oxygen just twice as freely as a flat slice of the same thickness exposed on both surfaces and 8 times as well as the same slice exposed on one surface.

Experiments were made on esculenta nerves suspended freely in Ringer solution to test this analysis. The nerves were of an average diameter of 0.075 cm. and by calculation required 1 per cent oxygen in the gas for the deepest fibers to be supplied. A descending series of oxygen concentrations from air through 10, 5, 2.5 and 1.3 per cent oxygen in different experiments had no effect on the total oxygen consumption per gram, but passing on to 0.5 per cent oxygen reduced the oxygen consumption to $\frac{2}{3}$. The general validity of these considerations may therefore be accepted.

It may also be noted that, so far as this evidence goes, any excess oxygen pressure above the necessary minimum does not increase the oxygen utilization. This is in accord with general experience; and Thunberg's (1904) finding that rabbit nerves had a higher metabolism in oxygen than in air may well have been due with larger nerves and higher metabolism, to incomplete oxygen supply in the latter case. A similar point arises in connection with Fenn's (1927) observations. He finds with dogfish nerves that stimulation causes only a 21 per cent increase in the resting oxygen consumption, whereas the present experiments indicate an increase of 100 per cent with continued stimulation. Differences of material, stimulation frequency, etc., render too direct a comparison of these results unwise, but assuming a considerable increase in oxygen consumption on stimulation the following point seems valid. Fenn's nerves were mostly above the critical diameter so that even at rest the innermost fibers were asphyxiated. During activity the outer fibers use more oxygen so that it penetrates even less completely into the nerve interior, and fibers that received oxygen at rest would no longer receive any, thus tending to diminish the increase of oxygen consumption observed.

b. *Anaerobic behaviour of nerve.* The extra heat produced and oxygen consumed by a nerve thrown into activity indicate that conduction is

ultimately dependent on oxidative processes, yet a nerve can still conduct after two or more hours in nitrogen. This could be explained in two ways: 1, the nerve contains a supply of oxygen or other reducible substances which can be drawn upon for an oxidative reaction essential to activity; or 2, the essential reaction is not oxidative but leaves behind products which must be ultimately oxidized, which can, however, accumulate for a time without destroying the mechanism. In either case, a nerve stimulated in nitrogen should develop an "oxygen debt" and when subsequently given access to oxygen would take up for some time an excess to restore the equilibrium. Similarly, since the active metabolism implies the continuation of resting processes as well, it may be expected that a nerve kept in nitrogen without stimulation will also take up oxygen more rapidly than its normal rate when this gas is again made available. If the basic reactions continued undisturbed in nitrogen for one hour, the nerve would have to take up later an excess of oxygen equal to 16 cmm. (for winter esculenta).

The experiments to determine this are by no means easy, for a relatively small effect must be measured in the presence of larger changes; and the results cannot claim great quantitative significance. Thirteen experiments have consistently shown, however, a definite excess in the oxygen uptake during the first hour that a nerve is exposed to air or oxygen after having been kept some time in nitrogen. Figure 5 shows the typical time course of the decrease in volume when air is passed through a vessel previously containing nitrogen. Controls with no nerve or dead nerve (boiled) show that equilibrium is reëstablished after the procedure within 15 minutes. When live nerve is present, however, the steady rate of oxygen consumption (indicated by the slope of the straight line) is not reached for ½ to 1 hour after air is admitted, up to this time there is a gradually diminishing excessive oxygen uptake. This excessive uptake is greater and appears to last longer when a nerve has been kept in nitrogen for two hours previously than when it has been kept for ½ hour. The total oxygen taken up after 2 hours in nitrogen averages 15 cmm. per gram more than the oxygen taken up after ½ hour in nitrogen. As far as this figure is significant, it would indicate that for two hours in the lack of oxygen a nerve can still support its intermediary metabolism at about ⅔ of its normal value.

It will be noted that the curve of oxygen uptake becomes very slightly convex to the X-axis just before the straight line is reached, indicating an oxygen consumption less than the steady rate. It seems probable, as also from other experiments, that after a period in nitrogen it requires some time in oxygen before the nerves fully reëstablish their normal oxygen consumption.

Similar experiments have been carried out on the effect of stimulation. Two sets of nerves were kept in nitrogen in the differential manometer for

one to two hours, the nerves on one side being continuously stimulated during this time. The action current showed the nerves to be still active, though rapidly failing, after two hours. When air was subsequently admitted to both sides at once, there was during the first $\frac{1}{2}$–1 hour a fairly regular displacement toward the previously stimulated side, indicating that it had developed a greater oxygen debt in nitrogen than had the unstimulated side. The short periods and uncertainty of equilibrium here, however, make the results suggestive rather than conclusive.

A nerve is able, therefore, to use up an oxidizing store or accumulate substances for later oxidization when deprived of access to oxygen itself. The latter mechanism might well be expected, by wide analogies in many tissues, to be found in a glycogen-lactic acid system. Experiments to be reported (Gerard and Meyerhof, 1927) have shown in fact that during rest in nitrogen a nerve produces considerable lactic acid as long as carbohydrate is available as precursor, though the carbohydrate is not resynthesized when oxygen is admitted. Stimulation does not significantly affect the lactic acid production.

The other mechanism might also be expected, however, from the known existence in tissues of intermediate oxygen carriers or hydrogen acceptors which are equivalent to "stored oxygen." If such an oxygen store were tapped to carry on activity in nitrogen, the usual oxidations would proceed and a nerve would continue to *produce* carbon dioxide (aside from breaking up preformed HCO_3^-). This would not be the case if only the lactic acid mechanism were present. Eight experiments, in which total carbon dioxide was determined by addition of strong acid at once to one set of nerves and in 20 hours to the companion set of nerves kept in nitrogen, showed an average carbon dioxide production of 1.5 cmm. per gram per hour. This corresponds roughly to 10 per cent of the total carbon dioxide production in oxygen for the whole period. Since any chemical reserves would be pretty well used up in 20 hours, it may be assumed that at first considerably more than $\frac{1}{10}$ of the normal oxidations are mediated through this mechanism.[7] (As further evidence: lactic acid production does not reach its maximal rate for some time after a nerve is put in nitrogen.) Technical difficulties made it impossible to determine whether or not an increased anaerobic carbon dioxide production resulted from stimulation.

Discussion. a. *Absolute values.* The absolute values found for resting and active oxygen consumption and carbon dioxide production are believed to be due to the true physiological behaviour (so far as any isolated tissue is physiological) of nerve. Disturbing effects have been evaluated and allowed for by extensive controls and conditions have been standardized as far as possible. The quantitative reliability of the method

[7] The rather remote possibility of the carbon dioxide arising from alcoholic fermentation is not excluded.

used has been amply demonstrated, and in these experiments the results are obtained with an error of only a few per cent.

Other workers have reported widely varying values for the gas exchange of nerve, from 17 cmm. per gram per hour to over a hundred times this value for resting oxygen or carbon dioxide exchange, and from 23 (or nothing) to 2,400 cmm. for the excess of activity. This work has been recently reviewed (Downing, Gerard and Hill, 1926; Davis, 1926) and will not be again gone over. It is worth noting, however, that three workers (Thunberg, 1904; Adam, 1921; Haberlandt, 1911) have used methods depending on pressure or volume changes, more or less similar to the one used here; and their results for resting oxygen consumption are in fairly good agreement with these and with each other, when allowances for temperature and other differences are made. All other methods gave considerably higher figures. The excess metabolism has not previously been measured manometrically, and the figures obtained by other methods have again been high—with the exception of Parker's, and the recent volumetric measurements of Fenn. Parker (1925) measured carbon dioxide production of dogfish nerve by an indicator method and found at 22°C. per gram nerve and hour, at rest 173 cmm. carbon dioxide, during activity an extra 29 cmm. of carbon dioxide given off. For frog sciatic the figures were 165 and 23, in both cases activity giving about a 15 per cent increase in production. Fenn (1927) finds an oxygen consumption in dogfish nerve, also at 22°C., of 81 cmm. per gram per hour and an increase on activity of 13 cmm., also about 15 per cent. Fenn's figures on frog nerve are not yet available, but he indicates (P. 343) an excess oxygen consumption on activity of 23 cmm. His results on resting carbon dioxide production of frog nerve (p. 337), assuming an R.Q. of 0.8, correspond to a resting oxygen consumption of 115, so that the increase on activity would equal about 21 per cent of the resting value. Comparing these with the present results, the resting metabolism of frog nerve seems a bit high, though not more than might be accounted for by differences in material, temperature, etc. The increase on activity, in absolute values, is almost the same as my value for continued stimulation; as per cent increase in the resting metabolism, however, my figure is 5 to 6 times as high as theirs. Some possible sources of discrepancy, aside from the different temperatures and frequencies used, have already been discussed.

The present experiments were performed under conditions as nearly as possible identical with those of the experiments on nerve heat production, and should therefore be directly comparable. The resting heat production of esculenta nerves, 2.0×10^{-5} cal. per gram per second, corresponds to an oxygen consumption of 14 cmm. per gram and hour (for burning carbohydrate; for fat about 7 per cent higher, and any food mixture somewhere between); the excess heat during activity, $6.9 \times 10.^{-5}$ cal. per gram per

second, corresponds to an oxygen consumption of 48 cmm. per gram and hour. *The agreement between the oxygen consumption calculated from heat values and the observed consumption,* (16 cmm. rest, 61 extra during activity)[8] *is so good that these two methods not only check one another, but it may be safely concluded that all the energy liberated as heat ultimately is derived from oxidations,* during activity as well as rest.

The R.Q. of rest, 0.77, is what might be anticipated by broad analogy with other tissues. During activity it approaches to or toward 1.0, so that the conclusion is suggested that in nerve as in muscle carbohydrate is the first choice of the machine. The important fact observed by Tashiro (1922) and confirmed by Winterstein and Hirschberg (1925), that a nerve gives off NH_3 during rest and in increased quantity during activity, must however find an explanation in the complete picture.[9]

It is interesting to note that the resting oxygen consumption of frog's nerve is almost the same as that of frog's muscle (18 cmm. per hour, variation 12 to 30—Meyerhof, 1920), whereas the increase during activity is only $\frac{1}{8000}$ as much. Only a few preliminary experiments on the influence of various chemicals and conditions on the resting metabolism have been made. Glucose in small amounts has no influence, in higher concentrations inhibits respiration. Sodium lactate acts similarly. Loebel (1925) and Meyerhof and Lohmann (1926) have found that glucose and especially lactate increased the oxygen consumption of central nervous tissue, or more especially prevented the rapid fall with time after isolation. The contained supply of oxidizable substance is enough to support the metabolism of isolated nerve through 20 hours, so addition of glucose would not be expected to have a great effect. The chemical processes of nerve metabolism will be considered more fully in another paper.

b. *The time course of the oxidations accompanying nerve activity.* It has been shown that the rise and fall of extra oxygen consumption does not entirely coincide with the start and stop of stimulation. The oxygen consumption increases at once, to be sure, but it continues to increase during the second 15 minute period as well; and the reverse effect obtains at the end of stimulation. Reasons have been given for believing that this is not an experimental artifact. It could not be due to fatigue, for this by decreasing oxygen consumption with time would give just the reverse effect.

[8] The quantitative heat experiments were all performed on winter frogs at temperatures betweeen 13° and 15°C. The values for oxygen consumption here given were obtained on winter and spring frogs at a temperature of 14°–7°C. which seems quite adequate to explain the somewhat higher figures for oxygen consumption.

[9] On continued stimulation at 280 shocks per second frog sciatics produce an excess of 4.5 mgm. per cent ammonia per hour at 15°C. (Gerard and Meyerhof, 1927). If all is due to the burning of protein, the protein would account for all the excess metabolism, also the R.Q. would be 0.95. It is, however, improbable that all the ammonia is produced by protein oxidation.

One is forced to the conclusion, therefore, that for 15 minutes (roughly) after conduction of an impulse a nerve continues to use extra oxygen: part of the delayed portions from the impulses of the first period adding to the oxygen consumed in the second, delayed portions of the second to the third, and so on, some delayed portions of the last period appearing after stimulation is stopped; hence the lag on rise and fall. It is only natural to associate this delayed oxygen consumption of activity with the known delayed heat of activity, especially as the latter, as shown above, is ultimately all oxidative. This is very interesting in relation to the behaviour of nerve in the absence of oxygen as shown by the heat production.

The heat of nerve activity falls with time in nitrogen and ultimately disappears, but at no stage is there any evidence that delayed heat falls faster than initial heat, or in other words, as long as a fiber is able to function, it continues to produce the usual two phases of heat. Conditions are very different in muscle where, in the lactic acid mechanism, the machine has a store of chemical potential energy which can be tapped to a considerable extent anaerobically (initial heat) and replenished by aerobic oxidations (delayed heat) at a much later time. *All the energy associated with nerve activity must be liberated during conduction and within the following 10 or 15 minutes.* The absolute amount of heat produced and the oxygen consumed during nerve activity shows that the energy used is all oxidative; the time course shows that much (possibly all) of the oxygen is consumed after conduction and corresponds to the delayed heat: there is nothing left over. It follows that *all the oxidations associated with the conduction of an impulse must also be completed within 15 minutes after the impulse has passed.* It is not impossible that the energy liberated during conduction itself is produced by anaerobic reactions—there is no certain evidence pro or con—but the oxidative restoration must follow at once. The chemical mechanism of conduction, therefore, is apparently not the familiar lactic acid one.

Other evidence pointing in the same direction is found in the facts that: 1) the ratio of delayed to initial heat in nerve is about 9:1, rather than 1.5:1 as in muscle and which corresponds to the 4:1 ratio of lactic acid removed to lactic acid burned, and 2) the rate of delayed heat production indicates a monomolecular rather than bimolecular reaction (Gerard, 1927a). More conclusive is the finding (Gerard and Meyerhof, 1927) that the resting production of lactic acid in nitrogen is not increased on stimulation. It is, of course, possible that lactic acid may be formed as an intermediary in the carbohydrate oxidation, but if so it is all burned and the series of reactions cannot be broken at the stage of lactic acid so as to permit its accumulation.

c. *The anaerobic activity of nerve.* It remains to account for the continued activity of nerve in nitrogen. There can be no possibility of dis-

solved oxygen remaining after the first few minutes, and it has been shown that a nerve develops an "oxygen debt" during rest, and still more during activity, in nitrogen. It has been also pointed out that such a debt might arise either by the accumulation of substances requiring oxidation or by the depletion of oxidizing substances. The first would permit the separation of activity from its oxidations and, although involved in the resting metabolism, can hardly be the cause of that activity. Nerve activity in nitrogen must depend on the presence in nerve of oxidizing substances.

Various types of indirect evidence favoring an "oxygen store" in nerve were presented by the Verworn school at the beginning of this century. Particularly Fröhlich (1904) and more recently and at greater length, Gottschalk (1914), studied the effect of varying exposures to oxygen on a subsequent asphyxia and concluded that a nerve is able to store oxygen. Objections raised against this thesis have not been experimentally grounded but were largely concerned with the "biogen" concept. The evidence presented in this paper is still indirect, but difficult to explain in other terms than an oxygen reserve. Precise knowledge of the nature of this reserve is still lacking, though the suggestion previously advanced (Gerard 1927b), invoking the oxidized stage of respiratory substances, still seems plausible. The chemical events of anaerobic and aerobic metabolism will be more fully considered in a later communication (Gerard and Meyerhof, 1927).

d. *"Fatigue."* The existence of "fatigue" in nerve has long been denied, but recent evidence seems to establish it beyond question. Tigerstedt (1912) and earlier workers showed the prolongation of the descending limb of the action current with continued stimulation; Field and Brücke (1926) have proven a tenfold lengthening of the absolutely refractory period during 10 minutes' tetanization; and a brief tetanization of 20 seconds has been shown (Gerard, 1927b) to modify the heat and action current produced by a second tetanization several minutes later. Findings now reported also fall in line.

The results of using different stimulation frequencies are especially interesting. It was shown previously (Gerard, Hill and Zotterman 1927) that, when tested by tetanization of a few seconds' duration, the heat and action current produced at frequencies of 280, 100, and 50 per second varied in the ratio of 1.0 to 0.7 to 0.5. Tests made in these experiments gave just the same ratios for the action currents produced by a brief tetanization. When the action current was measured, however, by breaking a continued tetanization at the various frequencies, the ratios became 1.0 to 1.0 to 0.6. The oxygen consumption at the same frequencies was also in just the same ratio. It is clear, therefore, that for short periods of activity after rest a nerve can respond to a high frequency of tetanization, over 100 per second, with greater activity than to a lower frequency; but

after some minutes of stimulation it can no longer follow the higher ones. These data are not yet complete enough for adequate quantitative treatment, but they are in good agreement with Field and Brücke's (1926) findings that after 10 minutes' stimulation the absolutely refractory period is lengthened to 9σ. Finally, a nerve tetanized continuously uses less than $\frac{1}{3}$ as much extra oxygen (per unit time of stimulation) as one tetanized for short periods with rest intervals between, whereas a nerve tetanized continuously still uses more than $\frac{2}{3}$ as much oxygen per hour at the end of several hours' stimulation as at the start.

These effects depend, therefore, very much more on the rate of activity than on the total amount. The nerve appears to strike an equilibrium within several seconds or minutes of the initiation of activity; for high degrees of stimulation it responds with a certain maximum rate of energy liberation, for lower degrees with a different rate, lower per unit of time but higher per unit of stimulation. This fall in energy liberating power of the nerve from the resting condition to the equilibrium level explains many of the phenomena regarded as signs of fatigue. It is doubtful if this usage is valid, and it would appear desirable to designate as "equilibration" or in some similar way this rapid adjustment, and to consider as fatigue only those further progressive changes induced by continued stimulation (especially in non-medullated nerve) which lead ultimately to inexcitability.

SUMMARY

1. The oxygen consumption of resting frog's sciatic nerve has been measured manometrically with the following results:

a. The conditions of preparation, within fairly wide limits, have no effect.

b. Aside from a variable excess during the first hour, the oxygen consumption continues constant for at least 20 hours.

c. Smaller (younger?) nerves have a higher oxygen consumption per unit weight than the larger ones. The upper and lower halves of one nerve respire alike.

d. The absolute value varies with species, season, etc. For *Esculenta:* in winter the average consumption (expressed in cmm. per gram of nerve per hour) at 14°C. was 16.0, variation 11 to 21; in spring, 21. For *Temporaria:* in winter, 23.0, variation 17 to 27; in spring, 28. The average variation between right and left nerves of one frog was about 10 per cent. These values agree very well with the resting heat production found for similar nerves.

e. Cutting, and still more crushing, a nerve produce a temporary increase. Killing with chloroform acts similarly.

f. Glucose and sodium lactate in small amounts have no influence; in

larger amounts they inhibit respiration. Potassium cyanide diminishes, but even in N/100 concentration does not entirely abolish oxygen consumption.

g. The temperature coefficient is 2.2 between 15° and 25°C.

2. The respiratory quotient of the resting metabolism is 0.77 for nerves from winter frogs.

3. The increased oxygen consumption and carbon dioxide production resulting from activity of frog's sciatic nerve have been measured by means of a differential manometer, with the folllwing results:

a. A nerve stimulated continually with 280 maximum induction shocks per second practically doubles its resting metabolism. The increased oxygen consumption continues above $\frac{2}{3}$ the initial value after several hours stimulation. When a nerve is stimulated intermittently, applying the same tetanus for 22 seconds every 4 minutes, the extra oxygen consumed is more than 3 times as much as when stimulation is continuous. The question of nerve fatigue is considered in this connection.

b. The absolute values of extra oxygen consumed are: for continued stimulation, average 18.0 cmm., variation 11 to 27; for intermittent stimulation, average 61.0, variation 48 to 91. Using intermittent stimulation the conditions of these experiments were very similar to those on heat production, and the agreement between extra heat produced and oxygen consumed on stimulation is good.

c. The excess oxygen consumption does not appear and cease sharply with the stimulation, but requires at least 15 minutes after stimulation is begun to reach its maximum and the same time or longer after stimulation is over to disappear. This lag is probably due to a delayed oxygen consumption corresponding to the delayed heat.

d. The temperature coefficient is 2.1 between 15° and 25°C. (1 experiment).

e. The respiratory quotient of the excess metabolism of activity is 0.97.

4. The question of oxygen diffusion into isolated nerves has been considered and a formula for the case of gas diffusion in a tissue cylinder presented. Oxygen consumption is independent of oxygen concentration when the latter is high enough so that all parts of the nerve have oxygen available.

5. A nerve kept in nitrogen develops an "oxygen debt," i.e., when subsequently given access to oxygen it takes up an excess during the first hour. The excess oxygen taken up by live nerve is greater after 2 hours than after $\frac{1}{2}$ hour in nitrogen, and is greater for live nerve than for a control with dead nerve.

6. During 20 hours in nitrogen a nerve produces (not merely gives off) about 10 per cent as much carbon dioxide as during the same time in oxygen.

7. The ability of a nerve to continue activity for some time in the abscence of outside oxygen is interpreted on the basis of these results as due to the presence in nerve of an oxidizing reserve. A lactic acid mechanism as a temporary source of anaerobic energy for activity seems to be excluded.

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THE OXIDATION OF SODIUM LACTATE BY RED BLOOD CELLS

GEORGE B. RAY

From the Department of Physiology, Western Reserve University School of Medicine, Cleveland, Ohio

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It is commonly believed that the adult red blood cell is without an appreciable metabolism (Harrop, 1919; Morawitz, 1909; Warburg, 1910, 1912). The recent work of Fürth and Lieben (1922) has shown, however, that under certain conditions of oxygen supply lactic acid is consumed by the red cells of the horse. The change noted in the lactic acid content of the solution in these experiments was far above the experimental error and is obviously concerned with the activity of the cells. As yet, however, no evidence of the gas metabolism of the red blood cell has been demonstrated. In this paper such evidence is presented together with certain possibilities as to the mechanism of the reaction.

The production of CO_2. The CO_2 produced in the reaction was determined in the manner devised by Osterhout (1919). The constant temperature modification, previously reported (Ray 1924), was used in these experiments. The solution employed was made by the addition of lactic acid to a sodium chloride solution and subsequently neutralizing with NaOH until the reaction of the solution was approximately pH 6.9. It is unfortunate that these experiments could not be carried out in a medium which was of the same hydrogen ion concentration as the blood, but any tendency toward alkalinity tends to interfere with the operation of the indicator method of measuring the rate of production of CO_2.

The red blood cells used in the majority of the experiments, and in all those which form the basis of the results given in this paper, were obtained from dogs. In some of the cases the animals were treated with heparin, while in other experiments oxalate was added to the shed blood. In a few of the experiments the blood was defibrinated by whipping. The method whereby coagulation is prevented appears to make no difference in the results. The cells were washed as soon as drawn and placed in the cold-room, from which they were removed only for the period of taking the sample. In the first experiments only cells less than 24 hours old were used. In the later experiments it was found that results of the same order could be obtained with cells 48 to 72 hours after bleeding. The fact that the cells after being in the cold for 72 hours reacted in a manner comparable

to the freshly drawn cells, seems to indicate that bacterial action had been kept at a minimum. The cells were suspended in 0.9 per cent NaCl. According to Yanagi (1924) the reaction of a suspension of cells in isotonic glucose is more nearly of the order of that found in whole blood. In the experiments to be reported it was felt that any substance capable of acting as a substrate for the cellular oxidation should be excluded in order that the effect on the sodium lactate might be clearly observed.

Preliminary experiments were carried out to determine the proper concentration of sodium lactate necessary to produce best results. It was found that in the lower concentrations of lactate little or no CO_2 was produced. In a concentration of M/12.5 the rate of production of CO_2 was rapid and this concentration was therefore adopted as the one used in further experiments. In all cases the concentration of the solution was made isotonic by the addition of NaCl and the reaction adjusted to pH 6.9.

Figure 1 shows the results of a typical experiment with washed cells.[1] The cell suspension, which was of approximately the same cellular concentration as the original blood, was placed in a tube and a current of air, free from CO_2, was passed through the solution in order that the hemoglobin might become thoroughly oxygenated. In certain other experiments this process was carried out in a tonometer, using room air, with comparable results. While the oxygenation process was going on the lactate solution was also washed free of CO_2. At the end of about 30 minutes the cells and lactate solution were mixed, placed in the reaction flask of the apparatus, and the rate of production of CO_2 determined as rapidly as possible. As may be seen from the curve, the rate of production remains constant over a considerable period of time. At the time marked *A* on the chart the blood had become dark in color, presenting the appearance of well-reduced venous blood. The reaction flask was therefore removed from the apparatus and a current of CO_2-free air passed through the cell suspension for 10 minutes. The color changes in the blood indicated that oxygen was taken up and upon placing the mixture in the apparatus once more an increased rate of production of CO_2 was found. This reaction was carried on for 7.5 hours, *B*, at the end of which time the blood was placed in the cold-room over night.

The treatment in the cold did not completely arrest the oxidation, for when the flask was removed in the morning, *C*, the hemoglobin was found to be reduced. There was also some indication of methemoglobin in the solution at this time, although no definite tests were made for this substance. Upon oxygenating the blood, in the same manner as on the

[1] Owing to errors in the previous calculation the readings in previous papers on the production of CO_2 are 40 per cent too high. This error has been corrected in this paper.

preceding day, the color once more approximated that of arterial blood. In placing the sample in the apparatus, however, it was found that the rate of production of CO_2, as may be seen from the curve, was very slow. This lack of oxidative activity was taken as the expression of the fact that the lactate content of the solution was exhausted. In consequence 10 cc. of CO_2-free lactate solution were added to the cell suspension, *D*. The result, as may be seen, was a sudden increase in the production of CO_2. This increase did not stay constant, but passed through a maximum and then started to decrease.

Experiments similar to the one given in detail were carried out and the results repeated 15 times. While there were minor variations in regard to the time at which the various events took place in the reaction there was a general agreement as to the activity of the cells. Experiments were also tried using cats' corpuscles in place of dogs', with comparable findings. It is believed, therefore, that under the conditions of these experiments red blood cells can oxidize sodium lactate. That the lactate comprises at least the most important portion of the substrate is shown by the fact that the addition of fresh lactate causes an acceleration in the rate of production of CO_2.

The question arises as to the other factors that might be responsible for the CO_2 produced during the reaction. That the activity is not a function of leucocytes is shown by the work of Levene and Meyers (1914), who found that leucocytes were incapable of oxidizing sodium lactate. It has been noted by Morawitz (1909), Warburg (1910) and Harrop (1919) that young cells have an appreciable metabolism. Rough microscopic examinations of the bloods used in the experiments showed that reticulated cells were few in number as compared with the adult cells. It was assumed, therefore, that these cells could not have produced the CO_2 observed in the course of the oxidation.

It will be noted that the blood pigments apparently bear some relationship to the oxidation of the cells, inasmuch as they undergo color changes which indicate the removal of oxygen. It is possible that these are the agents which bring about the oxidation by the cell. As has been mentioned there is some evidence that hemoglobin may behave as an oxidase. It is desirable, therefore, to test the reaction of hemoglobin in this respect and to compare its action with the action of the intact erythrocyte.

The hemoglobin used in these experiments was supplied by Dr. R. Ferry of Harvard Medical School and was made according to the method he has described (Ferry, 1923). The solution had a concentration of 0.126 gm. per cc. or 0.0075 molar (mol. wt. Hb = 16,700). The concentration of the lactate solution was the same as in the previously reported experiments with H_2O_2 (Ray, 1924) namely, M/50. The reaction, as in the case of the cells, was pH 6.9. The treatment of the reagents in these

experiments was exactly the same as in the experiments using corpuscles The lactate solution was swept free of CO_2 at the same time that the hemoglobin solution was oxygenated by a stream of CO_2-free air. The proportions of the reacting solutions were 25 cc. of the lactate solution and 1 cc. of the hemoglobin solution. The reading of the rate of production of CO_2 was made as soon as possible after the mixing of the reagents.

Figure 2 shows the results of this type of experiment. As in the preceding experiments the temperature of the reaction was 25°C. The points determining the curve are those found in 6 experiments. It will be noted that the rate of CO_2 production is of an altogether different type than that found in the case of the cell suspension. Instead of a constant rate of reaction, as evidenced by the constant rate of CO_2 production, there is a variable rate. At the start of the reaction the rate is rapid, but as the reaction goes on, there is a steady decrease until a rather slow rate is reached. The solution of hemoglobin is obviously not comparable to the suspension of red blood cells as regards oxidative activity.

On the other hand, the resemblance of the activity of the hemoglobin solution to the action of H_2O_2, under similar circumstances, is shown to a marked degree in figure 3 where curve *A* is the integral curve of the reaction between hemoglobin and sodium lactate, and curve *B* that for the activity of H_2O_2 under like conditions. As regards the general nature of the curves they are apparently of the same order. During the first stage of the reaction the results are practically the same and it is only during the latter part of the experiment that the amount of CO_2 produced by the hemoglobin exceeds the amount found in the case of the peroxide.

The consumption of oxygen and the products of the reaction.[2] While the production of CO_2 shown in the preceding section is definite, its significance as an index of metabolism is open to question. The well-known buffering

Fig. 1. The production of CO_2 from a mixture of red blood cells and sodium lactate. Explanation of the curve is given in the text.

Fig. 2. The production of CO_2 from mixtures of hemoglobin and sodium lactate. The points determining the curve are those determined in 6 experiments.

Fig. 3. The production of CO_2 from a mixture of hemoglobin and sodium lactate. This is the integral curve of figure 2. For comparison the curve of H_2O_2 under similar conditions is given in curve *B*.

Fig. 4. Changes in the O_2 and hemoglobin content of the reaction mixture (red blood cells and sodium lactate when air is excluded). Curve *A* represents the decrease in O_2 content; curve *B* the decrease in hemoglobin.

Fig. 5. Changes in the O_2 content and hemoglobin content of the mixture when air is admitted. Curve *A* is the consumption of O_2; *B*, the decrease in hemoglobin.

Fig. 6. The production of acetaldehyde in a mixture of red blood cells and sodium lactate when air is excluded from the reaction.

[2] The writer wishes to acknowledge the assistance of Mr. B. J. Wolpaw in carrying out certain of these determinations.

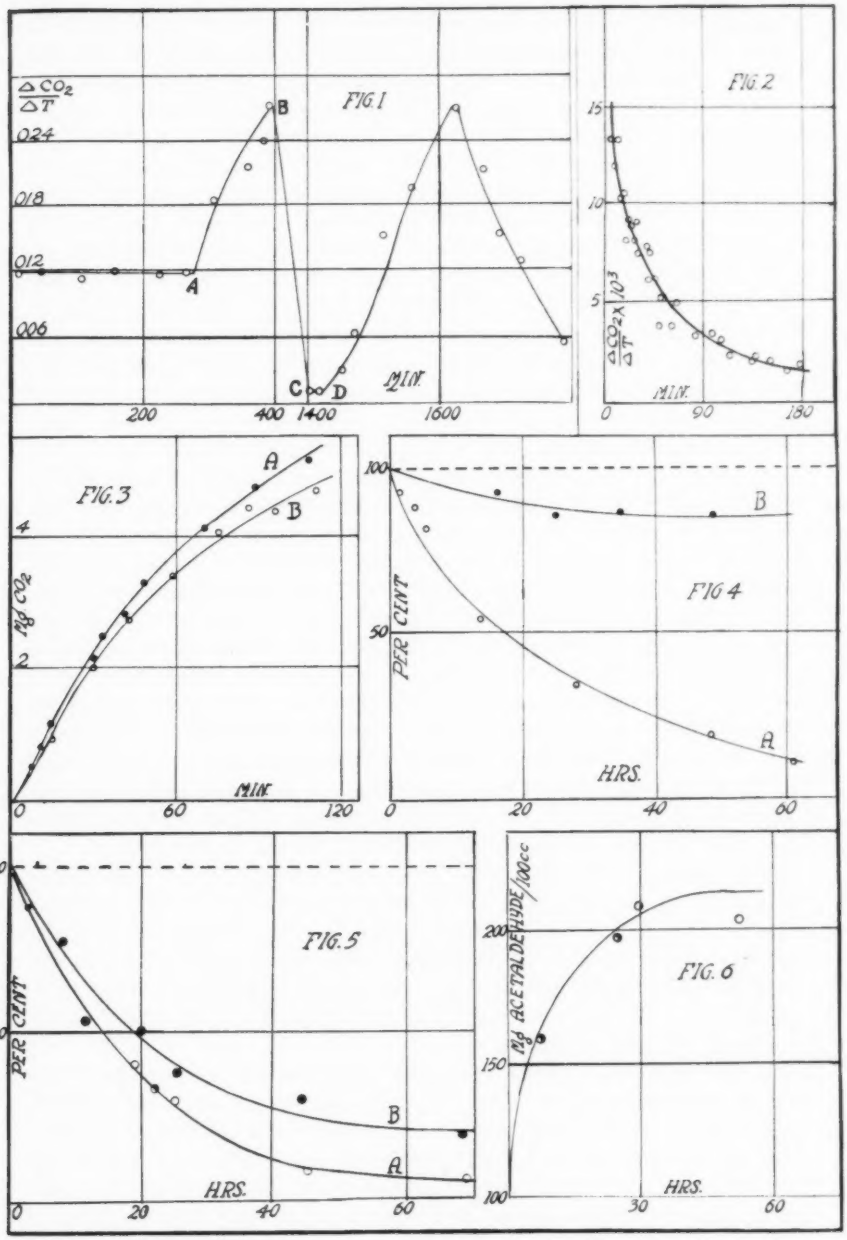
FIG. 1
$\frac{\Delta CO_2}{\Delta T}$
024
018
012
006
A
B
C
D
MIN.
200
400
1400
1600
FIG. 2
15
10
5
$\frac{\Delta CO_2 \times 10^3}{\Delta T}$
MIN.
0
90
180
FIG. 3
A
B
4
2
Mg CO_2
MIN.
0
60
120
FIG 4
100
50
PER CENT
B
A
HRS.
0
20
40
60
FIG. 5
100
50
PER CENT
B
A
HRS.
0
20
40
60
FIG. 6
200
150
100
Mg ACETALDEHYDE/100cc
HRS.
30
60

capacity of the red blood cell might possibly account for a part of the measured gas by releasing CO_2 in the period of the experiment. In order to prove definitely that the adult erythrocyte has an appreciable metabolism it is necessary to demonstrate a consumption of oxygen. The following experiments have been made in order to indicate that there is a well defined oxygen consumption under the conditions of the experiments.

The cells used were handled in a similar manner to those in the experiments upon the production of CO_2. The test solution was made up of M/12.5 sodium lactate with sufficient NaCl to make it isotonic; 200 cc. of the solution were placed in flasks and covered with parafin oil and sterilized for 10 minutes at 15 pounds pressure. The cells were added to the solution in a proportion of 50 cc. of cell suspension (of the same concentration as the blood) to 100 cc. of solution. Previous to the addition of the cells to the solution they were thoroughly oxygenated by a current of air.

Following the mixture of the cells and solution, the oxygen content was determined as soon as possible, which figure was taken for 100 per cent in the succeeding charts. Determinations were made at regular intervals. Inasmuch as the presence of methemoglobin had been suspected in the earlier experiments, determinations of the oxygen capacity and total blood pigment were made at each reading. The latter determination was made by the cyanhemoglobin method of Stadie (1920). Figure 4, shows the results of a series of experiments as outlined above. It will be noted that there is a steady decrease in the oxygen content of the solution (curve *A*) which continues over a period of about 50 hours. Curve *B* indicates the methemoglobin content of the reaction flask as determined by the difference between the hemoglobin, measured by O_2 capacity, and the total blood pigment. It will be noted that the change is insignificant and well within the error of the method. It is further significant that the color of methemoglobin, apparent in the first experiments, did not appear in these.

The reason for the failure of the reaction to behave as it did in the earlier experiments must be sought in the variation between the two modes of experiment. In the earlier experiments the blood was aerated by the passage of a current of air throughout the experimental period; in the present series, the solution was protected by a thick layer of oil. It is possible that the action of the air on the solution was the cause of the oxidation of the blood pigment.

Accordingly the experiments on oxygen consumption were repeated without the protecting layer of oil. Figure 5 shows the results of this series of experiments. Curve *A* is the oxygen consumption of the reaction, a curve that agrees quite well with that found in the first series. Curve *B* is that of the formation of the non-functional blood pigment. Instead of a negligible amount being formed we find that there is a large amount of hemo-

globin converted to the nonfunctional form. Obviously the presence of air is an important factor in the production of methemoglobin. That the non-functional blood pigment was methemoglobin was shown by the spectroscope.

Acetaldehyde as an intermediary product. The result of these experiments presents an anomalous situation. If the hemoglobin is the agent oxidizing the lactate one would expect that it would be reduced in the process. This is the case when air is excluded from the reaction. If, however, the solution has access to an abundant supply of oxygen we find that the hemoglobin itself is oxidized. Two possibilities suggest themselves as the causes of this change. One is that the hemoglobin is not the substance producing the reaction, and the other is that some substance is produced during the reaction that is capable, in the presence of atmospheric oxygen, of becoming a more potent oxidizing agent than the hemoglobin. In this case one might consider that hemoglobin induces the reaction but with the formation of products of the oxidation of the lactate one finds some substance produced that is capable of utilizing the oxygen of the air to form a compound of high oxidizing potential, which is able to carry on the reaction.

The question arises as to what this compound might be. In earlier experiments upon the action of H_2O_2 (Ray, 1924) under similar circumstances it was found that this condition of affairs existed and further examination pointed to the presence of acetaldehyde as the possible substance. Experiments were carried out to determine the presence of acetaldehyde. The method used was that devised by Stepp (1921) with the modification of first treating the sample with the Folin-Wu reagents to precipitate the blood protein and then treating in the usual manner. The method as used did not give very constant results, the variation being due without doubt to the loss of the extremely volatile acetaldehyde during the manipulation of the solutions.

The procedure of the experiments in this case was similar to those in the first part of the determination of oxygen consumption where the reacting mixture was covered with a thick layer of paraffin oil. It was thought that under these conditions there would be an accumulation of acetaldehyde if any was produced during the reaction. Samples taken from time to time showed that there was a measurable reduction of the silver and that acetaldehyde was present in the solution. These observations were checked by a qualitative examination using dimethylhydroresorscinol. In some experiments the odor of acetaldehyde was very distinct. Inasmuch as the results were so variable no attempt was made to average the figures found in all of the experiments. Figure 6 shows the results of one series of titrations in which the figures found were more consistent than in the usual experiment. It will be seen that there is a tendency for the acetaldehyde to increase in the system over a period of time corresponding to the

period of oxidation, as noted by the oxygen consumption. Control experiments were made in which the oil was omitted from the flask and in these no measurable amount of acetaldehyde was found to accumulate.

Discussion. The above results show clearly that there is a definite oxidation of sodium lactate by the red blood cell. Previous analysis of the action of the peroxide (Ray, 1924) has indicated that sodium lactate is oxidized to acetaldehyde by the action of the peroxide and that if no other source of oxygen is present the reaction apparently stops at this point. If oxygen is admitted then the acetaldehyde formed by the action of the peroxide is converted to acetyl peroxide which is capable of further oxidizing, the result of which is the production of acetic acid. From the experimental results reported in this paper we may suppose that a similar reaction takes place in the case of the red blood cell acting under similar conditions. When the reaction goes on with only the oxygen of the hemoglobin we find that total oxidation does not take place and that acetaldehyde accumulates in the system.

In the presence of the oxygen of the air no such accumulation is found, a fact which might be explained as being due to volatization were it not for the fact that hemoglobin is oxidized to methemoglobin during the course of the reaction. In order to explain this it is necessary to refer back to the action of H_2O_2. In these experiments it was found that the apparent peroxide content of the solution increased during the early stages of the reaction. This was found to be due to the formation of acetyl peroxide from the aldehyde by atmospheric oxygen. In an atmosphere of nitrogen this reaction did not take place. In the instance of the red blood cell the oxidation of the aldehyde manifests itself by the production of methemoglobin. This action coincides with the failure of acetaldehyde to accumulate, and indicates that the removal of this substance is the result of oxidation.

The course of the total reaction may be written as follows:

$$\text{I. } CH_3 \cdot HCOH \cdot COONa + HbO_2 \text{ (or } H_2O_2) \rightarrow CH_3 \cdot CHO + NaHCO_3 + Hb.$$

$$\text{II. } CH_3 \cdot CHO + O_2 \rightarrow CH_3 \cdot HC\begin{matrix} O \\ | \\ O \end{matrix} \text{ or } CH_3 \cdot HC{-}O{-}O{-}O{-}CH \cdot CH_3.$$

$$\text{III. } CH_3 \cdot HC\begin{matrix} O \\ | \\ O \end{matrix} + CH_3 \cdot CHO \rightarrow 2CH_3 \cdot COOH + H_2O.$$

or

IIIa. $CH_3 \cdot HC\begin{cases} O \\ | \\ O \end{cases} + CH_3 \cdot HCOH \cdot COOH \rightarrow CH_3 \cdot COOH + CH_3 \cdot CHO.$

IV. $CH_3 \cdot COOH + NaHCO_3 \rightarrow CH_3 \cdot COONa + CO_2.$

(In the oxidation of the lactate the step lactate to pyruvate has been omitted.)

As regards the exact rôle of hemoglobin in this oxidation it is not clear in what manner this substance acts. There is some evidence to indicate that red blood cells may exhibit an oxidase reaction as shown by the work of Kastle (1909), Lumiere and Chevotier (1905), and McClendon (1915). These qualitative observations are opposed, however, by the recent work of Conant (1923), indicating that there is no increase in oxidation potential when hemoglobin is oxygenated. Reed's observations (1916) on the oxidation of formic acid by platinum black indicate that an increase of the potential of the platinum gives an increase in the oxidation of the acid. One would expect from Conant's results that methomoglobin would be more capable of oxidizing the lactate than would hemoglobin. At present there seems to be no intimation of how this action may take place on the basis of the Bach-Chodat system of oxidation.

Recently, however, Holden (1925) has demonstrated the presence of glutathione in the red blood cell. It is possible that this substance, when allowed to act in conjunction with the oxygen of the hemoglobin, produces the oxidation.

SUMMARY

1. Adult red blood cells, when given a proper substrate, have an appreciable metabolism, as evidenced by the production of CO_2 and consumption of oxygen.

2. In the absence of atmospheric air the end product of the reaction is acetaldehyde.

3. It is suggested that in the presence of oxygen the acetaldehyde forms acetyl peroxide which in turn oxidizes the hemoglobin to methemoglobin.

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TEMPERATURE GRADIENTS IN THE TISSUES IN MAN

H. C. BAZETT AND B. McGLONE

From the Department of Physiology, University of Pennsylvania

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Measurements of the temperature gradients in the tissues have been made by many workers, but detailed data are few, and often little attention has been paid to possible technical errors.

Becquerel and Breschet (1835–1839) measured the temperature existing in the muscle of the upper arm in man, transfixing with a rod-shaped thermocouple (0.5 mm. diameter) of steel-copper or steel-platinum; the values for muscle temperatures were about the same as those for mouth temperatures, and 1.25° to 2.0°C. above those of the subcutaneous tissue. Since they observed similar temperatures with rods of 0.5 and 1.0 mm. diameter, they concluded that no error was present.

Claude Bernard (1876) using thermocouples determined arterial and venous temperatures in different parts of the body in animals, demonstrating varying temperatures according to locality. He carefully controlled the sources of error.

Lefèvre (1898, 1911) obtained data on temperatures in the tissues using a thermocouple made of steel tubing in needle form with a german silver core, and taking elaborate precautions to prevent heat loss from the needle. He did not consider the error due to the thermal conductivity of the metals relative to that of the tissues. He demonstrated the effect of cold baths, but gave few actual data. Hirsch (1903) and his co-workers noted low muscle temperatures in rabbits, but did not report any careful controls. Benedict and Slack (1911) demonstrated temperature gradients in the rectum and vagina to depths of 6 cm. Zondek (1919 and 1920) measured deep temperatures in man employing a fine mercury thermometer protected by a metal cannula (1.85 mm. diameter). Observations of the temperatures in the neighborhood of the femur were made; in patients at rest in bed at this depth temperatures a degree or more below that of the rectum were commonly observed, and the temperatures decreased peripherally; they were lower the more superficial or distal the position. He reported subcutaneous temperatures similar to those of previous workers. No figures are available in regard to possible errors due to inflammation or to heat loss by conduction along the cannula, though at the greater depths the latter error should be small. Interesting data are given of the effect

of surface thermal applications on the tissue temperatures. A profound effect of cold applications on the temperature of the brain was previously demonstrated by Leyton and Sherrington (1917) and thermoelectric observations by Macleod, Self and Taylor (1920), Macleod and Taylor (1921) and Taylor (1921) showed similar changes in deep temperature in many tissues as the result of local thermal applications. Moore and Quick (1924) have demonstrated the relatively low temperatures that commonly exist in the scrotum, and have pointed out the existence of a gradient within the peritoneal cavity in rabbits.

METHODS. For the measurement of tissue temperatures we have used thermocouples of steel hypodermic needle tubing with a constantan core; the latter was insulated by a single layer of silk and shellac. The needle tubing was about 13 cm. in length and of a size as indicated in the following table:

NEEDLE NUMBER	WEIGHT PER MILLIMETER LENGTH	EXT. DIAM.	THICKNESS OF WALL
	mgm.	*mm.*	*mm.*
23 and 40	0.55	0.35	0.074
21 and 24	0.79	0.46	0.077
11, 12, 13 and 14	1.11	0.51	0.100
22	1.35	0.57	0.108
25	2.77	0.88	0.138

The largest needles (nos. 22 and 25) were only used in preliminary experiments or in experiments to determine the error introduced by the thermal conductivity of the needle. The constantan core varied between 0.07 and 0.13 mm. in diameter, according to the size of steel tubing used, and was soldered to the bevelled end of the tubing; the actual junction extended probably over about 0.5 mm.

The needle (see fig. 1) was supported in a hard rubber cylinder, which in turn was contained in a brass cover, with an intermediate air space as a protection against air currents. At the lower end of the brass cover was a hard rubber cone, the tip of which alone touched the needle and the surrounding skin. A screw thread on the hard rubber cylinder allowed adjustment of the brass cover, and the length of needle protruding from the cover was thus altered and measured. A more detailed description of this thermocouple is given in another paper (Bazett and McGlone, 1927). At the fixed end of the needle the wires passed through rubber tubing, for a length of 1 to 2 meters, to a switch box; the constantan wire was continued without break, and the steel had no soldered junction except that with the steel needle at its fixed upper point. This lead consisted of tubing of the same steel as that used for the needle. The needle thermocouples were

held in a light spring clamp, or in a Bunsen burette clamp attached to a heavy stand with a universal boss-head clamp.

The general arrangement of the wiring employed in most of the experiments is indicated in figure 2. In this figure *TN* represents the needle thermocouple, *B* the switch box consisting of a hard rubber case, with parallel series of plugs and sockets, one series entirely of constantan and one of steel; each pair was connected to one thermocouple. The leads from a terminal pair passed to a standard couple, *SC*, maintained in a thermos flask in water; this couple was protected by paraffin wax. Any two of the constantan sockets could be connected by a flexible constantan wire with terminal plugs of constantan, *CP*. The circuit was completed by copper wire connected to steel plugs, *SP*, passing from the box to a variable resistance, *R*, (a post office plug resistance box), Ayrton shunt, *S*, and so to the galvanometer, *G*. The latter (Leeds and Northrup type 2500 A) had a resistance of 11 ohms, and the maximum sensitivity used has been a scale deflection of 2.2 mm. per microvolt when critically damped. Temperature differences have been estimated by galvanometer deflection. Both telescopic, and lamp and scale reading devices have been used, and finally photographic recording. The resistance of the two couples, leads and switch box has varied from 50 ohms to about 300 ohms. The sensitivity has been reduced, either by increasing the thickness of the galvanometer suspension, or by the introduction of extra resistance in box *R*, so as to give any desired temperature factor (usually between 0.3 and 1.0°C. per cm.).

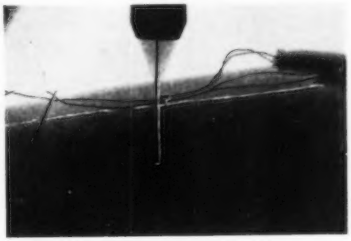

Fig. 1. X-ray photograph (⅓ and retouched), showing needle thermocouple introduced vertically into the forearm on its extensor aspect to a depth of 20 mm. (subject M). The elbow is slightly flexed. Two loop thermocouples (of light wire) are located in the subcutaneous tissue proximal to (to right of) the needle at depths of 5.2 and 6.0 mm. These were drawn into place after transfixing the skin obliquely with needles (seen on the left). The boundary of fat and muscle may be distinctly seen at about 6.5 mm. depth. Retouching has exaggerated the thickness of the wire loops.

In some experiments designed to test errors, where rapid observations were necessary, the switch box was replaced by a special rotatory switch (Leeds and Northrup—10 point enclosed switch no. 8240), which, however, necessitated copper-constantan connections. All of these as well as those of steel with copper, were insulated with rubber tubing and immersed in one large thermos flask filled with paraffin oil.

The thermos flask with the standard couple was in many experiments kept at room temperature and the water was stirred by bubbling moist air

through it; its thermometer was read frequently. In some experiments the thermos was kept within an incubator, so that the change of temperature throughout the course of an experiment was reduced to a few hundredths of a degree; in this case stirring was not required.

Some of the sockets on the switch box were connected to iron-constantan couples for surface and rectal temperature measurements. The surface thermocouple consisted of double strands of wire (0.3 mm. diameter); it was insulated by a single coat of shellac and protected from air currents by a light asbestos cover of 5 to 10 mm. diameter. A later model had only a single strand of each metal bent into a reverse loop (see fig. 2, *TS*), so as to surround the needle at its entrance into the skin; this type was protected from air currents by a small linen shield. Rectal temperatures were taken in a few experiments with a thermocouple enclosed in a rubber catheter, with the junction opposite the aperture. Iron wire was sometimes used for the surface, rectal and standard thermocouples owing to difficulties arising from rusting within the steel tubing.

In estimating errors, knowledge of the temperature of the brass cover was essential. It was measured with the surface thermocouple, or by special thermocouples built within the brass covers to allow a more exact determination of the temperature within this space.

To provide an instrument of lower thermal conductivity and capacity, thermocouples have also been made from constantan and manganin wire (0.08 mm. diameter); the manganin was soldered to the constantan 3 to 5 cm. from its free end forming a Y-shaped loop. For determining tissue temperatures the free end of the constantan was threaded through a needle of 0.30 to 0.35 mm. diameter. With these the tissues were transfixed, and by traction the junction was drawn into position. The depth of these thermocouples was estimated by x-ray photographs. These junctions also serve excellently for surface temperature measurements. We are indebted to Dr. W. P. White of the Geophysics Laboratory of the Carnegie Institution at Washington for valuable suggestions during the development of this thermocouple.

The needles were sterilized in alcohol before insertion, and the loops, which were insulated with a thin coating of shellac, by immersion in 10 per cent formalin, and washing in boiled water. After each experimental day the loops were soaked in alcohol, dried and reshellaced.

The temperature factor was determined by empirical standardisation at two temperatures against certified mercury thermometers, almost invariably once during the course of each experiment, though occasionally standardisation was repeated. A linear relationship between temperature difference and galvanometer deflection has been assumed; any apparent shift of the temperature zero has been attributed to parasitic currents and has been included in the calculations.

Mercury thermometers were also used to read the room temperatures (dry and wet bulb), calibrated half-minute clinical thermometers for urine temperatures, and kata thermometers (dry and wet bulb) to estimate the degree of air movement.

The temperature measurements recorded in this paper were largely de-

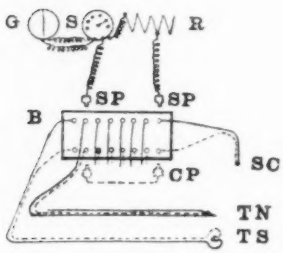

FIGURE 2

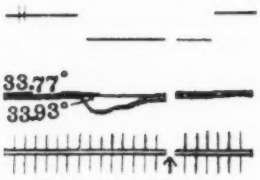

FIGURE 4

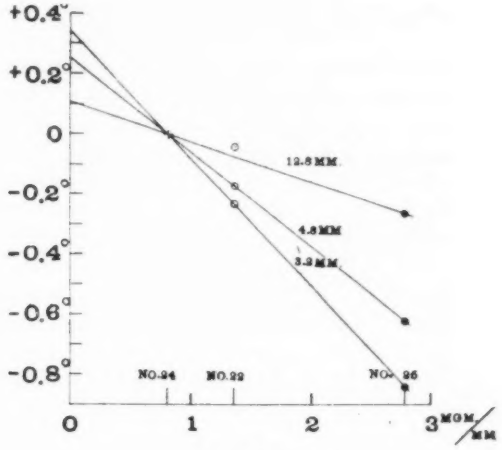

FIGURE 3

Fig. 2. Diagram of wiring, as described in text. Steel or iron wire is indicated by solid lines, constantan wire by interrupted lines, and connecting copper leads by spirals. The arrangement of the interchangeable plugs is that for completing the circuit when the thermocouple needle, *TN*, is in use.

Fig. 3. Differences in the apparent temperature according to the size of the needle thermocouple employed. Needles nos. 22 and 25 are compared with a smaller needle no. 24. The amounts by which the temperatures of nos. 22 and 25 differ in degrees centigrade from that of no. 24 are plotted as ordinates and the mass in mgm. p. mm. as abscissae.

Fig. 4. Effect of a small cover of gauze on surface temperature. Two constantan-manganin thermocouples (wire 0.08 mm. diameter) were employed, one lying on the surface, and the other imbedded in the dermis directly beneath it at a depth of 0.7 mm. Photographic records were obtained from two galvanometers with a sensitivity such as 1 mm. was equivalent to 0.1°C. The darker and broader record represents the surface (initial temperature 33.77°) and the other the deeper (initial temperature 33.93°). Time is recorded below in seconds. The signal indicates the period of application of a gauze cover; the interval omitted (vertical arrow) in reproduction is 35 seconds. Dry bulb temperature 24.1°, wet bulb temperature 19.6°, dry kata-thermometer reading 3.1 m.c. per sq. cm. per second, wet katathermometer 13.2, effective temperature 21.7°. (In subsequent descriptions these conditions will be designated as D.B., W.B., D.K., and W.K., respectively.) Record reproduced ½.

termined by the use of needles; the loop type of later development has served mainly to check the accuracy of the other method. As the experiments have continued during several years the details of technique have been subject to improvement.

Subjects used. Determinations of temperature gradients in the forearm have been made on 14 subjects, 10 male and 4 female; a single series of observations was made on 4 subjects, on others as many as five series have been made. On the authors a large series of observations has been obtained. With the few exceptions that will be noted the forearm has been bared and exposed to the environmental conditions for half an hour to two hours prior to the first temperature measurement. Twenty-seven determinations of the gradients in the thigh have been made, but only on one subject. The effect of swimming in sea-water at 20° to 21° has been investigated on 10 occasions, employing 3 male and 3 female subjects.

The bulk of these data has been obtained on the authors, and, although the corrected values for tissue gradients may have slight inaccuracies, the numerous surface and urine temperatures are not subject to such errors. Although the authors have been resident in the same geographical area for about 5 years, one (B) has been previously accustomed to the colder climatic conditions of England, and the other (M) has had no marked change of climate. Conditions feel neutral to B, when cool to M, and warm to B when neutral to M. Of the two subjects B is the thinner (7 per cent below normal Hassing, Tabulae Biologicae 1926), and M the fatter (6 per cent above normal on the same scale). On the forearm subject B appears to have a covering of fine hairs approximately $2\frac{1}{2}$ times as numerous as those of M.

Estimation of errors. A comparison of the surface temperature as determined by a loop thermocouple and that of the tissue at 2 or 3 mm. depth by a needle thermocouple introduced vertically presents an apparent discrepancy; the surface temperature often appears to be higher. This indicates probably a systematic error in the reading of the needle thermocouple. The discrepancies are more evident the colder the room, the greater the temperature gradients, and the greater the cross sectional area of the needle. The error appears therefore to be due to the high thermal conductivity of the steel, which causes a flow of heat from the tissues to the needle. To determine the order of magnitude of these errors a series of experiments of different types was conducted.

As the result of these experiments the following empirical formula was found to give an approximate estimation of the error.

$$E = \frac{KA\,(\theta_i - \theta_B)}{l^n}$$

where E represents the error in degrees centigrade, K is a constant with an approximate value of 0.46, A is the cross sectional area of the needle tubing estimated by weight and expressed in mgm. per mm. length; l is the depth in mm. to which the junction is inserted, and its exponent n has a value of about 1.25; θ_l is the apparent temperature at depth l; θ_B is the temperature of the brass cover of the needle holder.

The experiments, by which this formula was derived and tested, were as follows: the formula itself was derived mainly from comparison of data obtained with needles of different cross sectional area inserted in the human thigh. In all the experiments, difficulties arose as the result of chance errors due to anatomical structures, physiological changes (e.g., inflammation), and variations in environmental conditions, as will be noted in an example to be described later. However, the general effect of the cross sectional area of the needle on apparent temperature could be demonstrated and may be seen in figure 3, where the average temperature differences between three needles for depths of insertion of 3.2, 4.8 and 12.8 mm. are plotted in relation to the weight of 1 mm. length of needle; all the observations were made the same day with a room temperature between 23° and 24°C. By extrapolation of the apparent straight lines one might infer that on this occasion the error of needle 24 varied between 0.34° at 3.2 mm. depth and 0.11° at 11.8. For these two depths the errors calculated by the formula are 0.48° and 0.12°, the agreement being poor at the lesser depths, since the formula derived from this and similar experiments has been modified to give a better agreement with the values obtained by other methods.

In the experiment plotted in figure 3 where the environmental conditions were relatively uniform, the systematic error appeared to be inversely proportional to the depth of insertion and might be calculated according to the following formula: $E = \frac{KA}{l}$, where K is a constant, approximately 1.24. But when the environmental conditions also vary, the error is clearly some function of the temperature gradients. To allow for this, the factor $(\theta_l - \theta_B)$, or the difference in temperature between the two ends of the needle, has been introduced into the formula, since this difference must be concerned in the loss of heat along the needle (even though the gradients along the needle cannot be regular). Although a simple linear relationship of the error to this temperature difference has been assumed, a complicated relationship is more probable. Since θ_B is relatively constant, the value $(\theta_l - \theta_B)$ varies with the depth of insertion; if $(\theta_l - \theta_B)$ be introduced into the numerator of the equation a similar function of l (approximately $l^{0.25}$) must be introduced into the denominator. By graphical methods and on empirical grounds the formula was chosen as one giving reasonable values in most of the test experiments. The constants can only be considered valid within a limited temperature range.

The data obtained in the experiments with needles of different size also illustrate physiological variations to be encountered in the tissues even with thermocouples less than 2 cm. apart and are described in more detail. In these experiments the two needles were inserted radially and close together on the middle of the inner surface of the thigh (over the sartorius muscle), but even so it was found difficult to find positions having comparable temperatures. If the points of entrance of the needles lay in a line parallel to the long axis of the limb, the more proximal needle indicated the higher temperature, if in a line at right angles to the axis, the inner needle gave the higher reading with equal depths of insertion. Fairly comparable points were obtained when the needles were inserted in a line drawn obliquely across the thigh so

that the proximal needle lay somewhat to the outer side (designated in the figures *O.U.—outer and upper*). Even then in order to get a true comparison the position of the two needles had to be reversed and a second series of observations made. The comparison therefore of three needles as in the experiment illustrated in figure 3, required a series of eight punctures, and during this time physiological changes due to inflammation or environmental conditions might occur.

These points are illustrated by figures 5 and 6, the results of a single experiment, the former giving the apparent values as directly observed and the latter those obtained after correction. They demonstrate the order of magnitude of the error

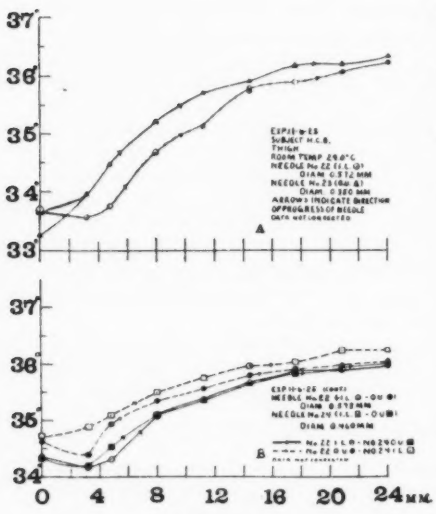

FIGURE 5

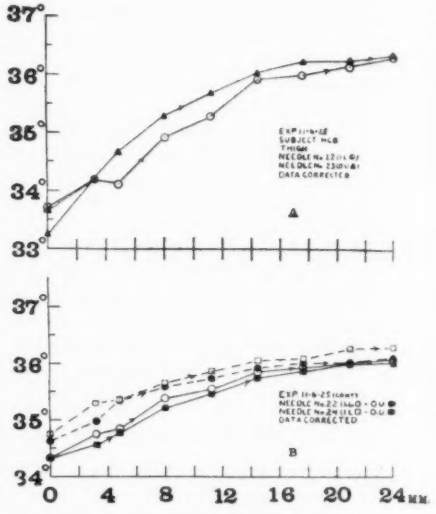

FIGURE 6

Fig. 5. Apparent temperatures at different depths under the conditions as indicated in figure and in text. The upper chart (A) represents the earliest of a series of observations. Needle no. 22 is indicated by circles, needle no. 23 by triangles and needle no. 24 by squares. Open symbols are used for the inner and lower (I. L.) position and closed symbols for the outer and upper (O.U.). Temperatures measurements of the same series are joined by lines of a similar type.

Fig. 6. The same observations of figure 5, corrected according to the formula and plotted.

correction. Figures 5 A and 6 A represent the gradients observed in the thigh before the subject had attained equilibrium after entering the warm room, and show steeper gradients than those observed when the subject had been exposed for a longer period to the high environmental temperatures. Only two punctures were made but the positions appear to be relatively comparable. In figures 5 A and 6 A two values are plotted for the surface temperature at the *O.U.* position, the lower obtained at the beginning of the experiment and the higher on its completion. The temperature gradients illustrated in figures 5 B and 6 B were observed approximately $\frac{3}{4}$ hour (indicated by solid lines) and $1\frac{1}{2}$ hour later (indicated by interrupted lines) and it

will be observed that during the whole time the surface was rising (approximately 1°C.); during this same time the deep temperature had shown an initial fall of about 0.3°, and had then remained steady or risen slightly. During the same time the urine temperature also fell 0.17°C. As the result of the irritation of the needles a slight inflammatory reaction could be distinguished in the surrounding skin during the latter half of the series of observations indicated in figures 5 B and 6 B by the solid lines, and continued throughout those indicated by the interrupted line. The differences due to anatomical variations are particularly noticeable at a depth of 3.2 mm. in figures 5 B and 6 B where the average recorded by the two needles in the inner and lower position is considerably higher than that similarly observed in the upper and outer position; it will also be noted that in the series indicated by the interrupted line there appeared to be some anatomical variation causing an increased temperature at the greater depths in the inner and lower position. Slight variations in the relationship of the needles to blood vessels is likely and occasionally differences of this type have been noted when hemorrhage under pressure along the needle track has afforded evidence of the actual puncture of a small artery. In spite of all the above difficulties it will be seen that the means of the corrected values for needle 22 (fig. 6 B) are very close to the corresponding values obtained with the smaller needle 24.

Two experiments were made on man, and several on animals, comparing the apparent temperatures of vertical and oblique needles; the positions of the junctions were determined by x-ray. Examples are given in table 1 illustrating the application of the above formula to data so obtained; for comparison values are here expressed to 0.01°C. It is unlikely that the oblique needle had any serious error, since it was inserted to a distance of over 20 mm., and owing to the warming of the brass cover by contact with the skin the temperature differences between the two ends of the needles were small. The general agreement of the two sets of figures is fairly good.

A slender mercury thermometer was introduced into the thigh muscles of an anesthetised dog and a needle thermocouple was located adjacent and parallel to it. The thermojunction was initially situated slightly deeper than the end of the bulb and was withdrawn in steps. X-ray photographs were taken to determine the relative positions. The mean temperature of thermometer during this time was 37.85°, a temperature of 0.46° below the mean rectal temperature; the thermocouple readings when corrected gave a mean value of 37.81°, a better agreement than was anticipated since no attempt was made to integrate the temperature curve.

It was possible by using a special needle as described to warm the brass cover by means of a resistance coil wound round it, until the temperature within the cover was identical with that recorded at the needle tip so that no withdrawal of heat from the tissues might occur. During the time the needle was being heated the temperature of the tissues might be falling, and consequently comparison was made with a second needle inserted to the same depth about 15 mm. distant. In one experiment the error so determined at 21 mm. depth, was 0.11° and by calculation 0.09°, and in another at 4.4 mm. depth 0.37° and by calculation 0.38°. This method, while simple in theory, is difficult in practice, since the condition of the tissues may be modified by heat derived from the warm needle cover.

The validity of the formula was also tested by means of agar jelly in which a temperature gradient was created from below upwards. The jelly (1 per cent) was contained in a brass box heated below by a water bath and protected from cooling on the sides by asbestos and hard rubber coverings; the surface was cooled by conduction and evaporation from exposure to the room air. Into this jelly needles were introduced more than 20 mm. horizontally; this excluded any appreciable tem-

TABLE 1

Data with needles 1.11 mgm. per mm. length

NUMBER	SUBJECT	DATE	POSITION	DIRECTION	l	θ_l	θ_B	E	CORRECTED TEMPERATURE	SURFACE TEMPERATURE	REMARKS
					mm.	°C	°C.	°C.	°C.	°C.	
1	B	June 17, 1925	Forearm	Oblique	23.2	34.56	32.2	0.02	34.58	35.0	Room as in experiment 2.
			Forearm	Radial	4.0	34.05	29.5	0.41	34.46	34.3	Urine 37.0°
2	M	June 17, 1925	Forearm	Oblique	21.6	34.98	32.8	0.02	35.00	34.3	Room 26 to 27°C. Wet bulb 16.6 to 18.2°
			Forearm	Radial	3.2	34.40	29.6	0.58	34.98	34.4	Dry kata 2.74. Wet kata 12.10. Urine 36.4°
3	Dog	May 15, 1925	Thigh	Oblique	30.0	36.80	31.0	0.04	36.84	35.0	Ether-veronal. Rectal
			Thigh	Radial	13.6	35.75	31.4	0.10	36.85	34.1	38.53°
4	Dog	May 27, 1925	Thigh	Oblique	30.0	34.71	28.9	0.04	34.75	31.5	Ether-veronal. Rectal
			Thigh	Radial	12.0	34.61	28.5	0.14	34.75	31.7	35.88°
			Thigh	Oblique	28.4	34.37	28.1	0.05	34.42	31.5	Rectal 35.75°. Radial needle 2.5 mm. superficial to oblique by x-ray
			Thigh	Radial	8.8	34.30	28.0	0.21	34.51	31.5	
			Thigh	Oblique	25.2	33.84	28.2	0.05	33.89	31.4	Rectal 35.75°. Radial needle 1.5 mm. superficial to oblique
			Thigh	Radial	9.6	33.60	27.9	0.17	33.77	31.6	

perature gradient. Other needles were inserted vertically along the gradient as in experiments on the tissues. The apparent temperature of the vertical needle was always the lower. In many cases the observed difference between the two needles was within 0.1°C. of that calculated by the formula adopted for use in the tissues.

In our experiments of recent date two loops have been introduced at depths of 3 to 6 mm., as near as possible to a needle introduced radially, and their spatial relationships determined by x-ray photographs, one of which is reproduced in figure 1. In four experiments which are described in this paper the mean difference between the corrected needle values and those determined by the loops at these depths was 0.03°. When the room temperature was below 20° the discrepancies were greater; in subject B the difference in temperature between the needle point and needle cover was great, consequently the factor was multiplied by a large value, and the error appeared to be overestimated; in subject M the tissues were extremely cold, their own thermal conductivity possibly very low, and the error appeared underestimated (see fig. 9). In either case an error of 1 mm. in estimating the relative depths would be sufficient to account for the discrepancies.

In the measurement of *surface temperatures* it is impossible to employ wires in contact with the skin for some 3 cm., as in the standard method of Benedict (1925), since our experiments were concerned with temperatures at points only. The accuracy of the methods used must therefore be considered. The factors which cause errors in surface measurements are: 1, loss of heat along the wire; 2, the assumption of an intermediate temperature by the thermocouple when this lies at the junction of skin and air, and 3, a diminution in heat loss sufficient to cause an abnormally high surface temperature if a cover is used to raise the temperature of the air above the thermocouple. The effect of these factors was estimated by measuring the surface temperatures with thermocouples of wire of varying gauge, with or without shielding. If the wire were of small gauge identical temperatures were obtained, and a shield had little effect. It is probable that there was no error greater than ± 0.1°C. The following considerations together with the data of the accompanying paper have led us to this conclusion.

As far as the first source of error is concerned, wires bent into the special shape previously described have been used for many of the readings, so that a length of about 10 mm. of each element was in contact with the skin, and yet in proximity to the point of entrance of the needle. The error due to thermal conduction should therefore be somewhere near that observed with needle tubing of 0.35 or 0.46 mm. diameter when inserted to a depth of about 10 mm. and should be well under 0.1°C. By comparison it may be noted that the thermal conductivity of the large copper wire couple used by Benedict (1925) would be over 20 times that of the wire employed by us; notwithstanding its larger surface contact it might have as great an error. At room temperatures of 18° or less, this error is measureable; when a surface couple of wire of 0.3 mm. diameter is moved into position, even though it has been previously warmed on an adjacent area of the skin, a slight but definite fall of temperature may be noted in the tissues several millimeters deep to it.

In regard to the second source of error the thermal conductivity and capacity of the tissues is so great relative to that of the air, and the air in contact with the surface of the skin is so warm, that although an intermediate temperature is assumed

by an exposed thermocouple, this temperature will be quite close to that of the surface; this will be particularly true if the wire used is of such a diameter that, when tension is applied, it lies within a groove. To diminish this error the heavier junctions were often shielded by a small linen cover placed over them about ½ minute prior to the observations. In the earlier types with double wires the light asbestos shield served the same purpose. With the fine wire manganin-constantan thermocouples this error is not appreciable.

The third error is one in the opposite direction since any shield will cause the temperature to rise to some degree above its normal level. If the reading can be taken quickly as in Benedict's experiments, this should be practically negligible, but surface thermocouples cannot be adjusted rapidly around the needles. The effect of the coverings used or of their absence was demonstrated in experiments of the following type. The light constantan-manganin thermocouples under slight tension lie in a furrow in the skin. If now a cover of 2 layers of gauze (3.0 by 1.5 cm. in size) be warmed on an adjacent region of the skin and be moved upon the surface thermocouple, an initial fall of surface temperature is seen (see fig. 4) with a quick return to the previous level, which is then maintained. A cover of this size is cooled while being moved through the air, hence the initial fall of temperature. There is

TABLE 2

Environmental conditions, urine temperature and surface temperature of forearm

SUBJECT	WARM			COLD—SHORT EXPOSURE (2 HOURS)			COLD—LONG EXPOSURE		
	Urine temperature	Surface temperature	Urine – surface temperature difference	Urine temperature	Surface temperature	Urine – surface temperature difference	Urine temperature	Surface temperature	Urine – surface temperature difference
B.....	37.25	33.66	3.59	36.47	28.79	7.68	36.86	30.61	6.25
M.....	36.88	33.23	3.65	36.58	27.58	8.00	36.40	27.32	9.08

evidence that a shield of this size and nature raises the skin temperature slightly after some 30 to 40 seconds, but the change is less than 0.1°.

The most important source of error is the thermal conductivity of the metal wires. If a needle of fine bore be pressed onto the skin so as to make a groove of 15 to 20 mm. length, although uncovered it may give a reading identical with that given by a covered thermocouple.

RESULTS. *Surface and urine temperatures under the conditions of the experiments.* When the two authors are compared on the same days under identical conditions the average results given in table 2 were obtained. The table demonstrates that both the surface and urine temperatures of subject M are consistently lower than those of B, and these differences are more marked after long exposure to cold; with short exposures to cold the urine temperatures of M rise temporarily above those of B.

Many attempts have been made to correlate our data on *surface temperatures* with the physical conditions of the environment. The surface temperatures showed only poor correlation with either dry or wet bulb

temperatures, and the correlation was not improved by using katathermometer determinations of cooling power. The data were plotted against the empirically derived "effective temperature index" (basic scale) of Yagloglou and his co-workers (Houghton and Yagloglou, 1923 a, and b, 1924), Yagloglou and Miller, 1925), the katathermometer readings only being employed for the calculation of the amount of air movement (Hargood-Ash and L. Hill, 1923) in determining the effective temperature. Confirming McConnell and Yagloglou (1924), the basic scale (determined for subjects stripped to the waist) agrees fairly well with surface temperatures of bare areas; the agreement is not so good with the scale given for subjects fully clothed. The correlation of the surface temperatures of the authors with the former scale is shown in figure 7 (A and B); the surface temperatures of the two subjects are similar at the higher effective temperatures, but those of subject M fall to a greater extent at the lesser effective temperatures.

In figure 7 each point plotted represents the mean value for one experimental day, except when there has been a progressive change during the day. For instance, the four values plotted for subject M at an effective temperature of 12.8°, which lie below the mean level, represent a progressive cooling of this subject after immobilisation, and correspond with the two values lying near the mean level, which were obtained a little earlier directly after the fixation of the arm. These represent two separate experiments at this temperature; after immobilisation the subject appeared unable to maintain thermal equilibrium, although previously the temperatures had not been greatly altered in spite of a long exposure to the same conditions. When shorter periods of exposure were employed (e.g., those at 7.2° effective temperature) little difference was seen between the two subjects. Many determinations of the surface temperatures of the inner side of the thigh have also been made on subject B, when seated for several hours without trousers, though otherwise clothed. At the higher effective temperatures (about 22°) the temperature of the surface of the thigh was similar to that of the forearm; at lower effective temperatures (about 18°) it was consistently lower (about 1.0°).

Any exact definition of a comfort zone is not possible except on an individual basis; where the data have been averaged for conditions of warmth and cold, as in table 2, conditions above 17° on the effective temperature scale have been considered as warm, and those below this limit as cold.

The changes in urine temperature are complicated by effects of immobilisation, and by diurnal variations, as well as by environmental conditions. During the first few hours in a cold room the urine temperatures were maintained or slightly increased, but were decreased later particularly in subject M. On the return to a warm room the urine temperatures fell abruptly; the fall was greater and more prolonged in subject M than in B. The temporary rise of urine temperature on exposure to cold was more noticeable in subject M when he was immobilised. In a warm room there was a rise of 0.5° in both subjects during the course of the experiment.

Tissue gradients in the forearm in subjects B, M and L. Data have been

obtained on subjects B and M under identical environmental conditions and after the bare forearm had been exposed for at least two hours. The temperatures observed are plotted in figure 9.

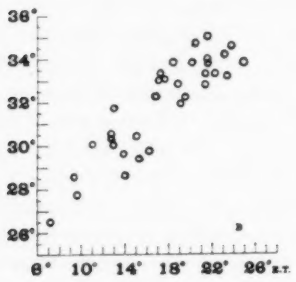

FIGURE 7A

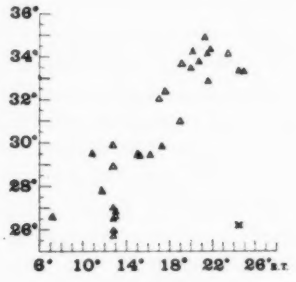

FIGURE 7B

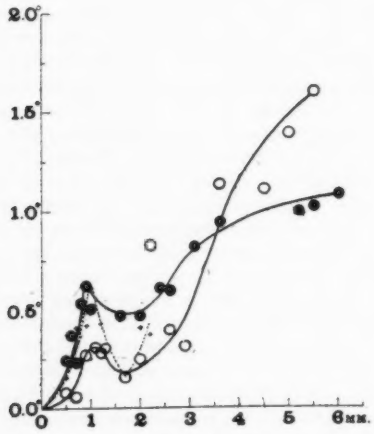

FIGURE 8

Fig. 7. A. Surface temperature of forearm of subject B (O) plotted as ordinates against the "effective temperature" index (basic scale) as abscissae.

B. Data obtained on subject M (△) plotted in similar manner.

Fig. 8. Composite curves representing probable gradients in subjects under environmental conditions greater than 17° E.T. (solid circle), colder than 17° E.T. (open circle) and with a local hyperemia of the inflammatory type without regard to room conditions (crosses, joined by interrupted line). The temperature between the surface and a point in the dermis or subcutaneous fat is plotted as ordinate against the depth of the thermocouple as abscissa. (The discrepant value at a depth of 2.2 mm. represents a single experiment in which the thermocouple may have been adjacent to an artery.)

On January 12 subject B was examined 4 to 5 hours after entering the cold room, and 1 to 2 hours after a 20 minutes' absence for lunch in a slightly warmer room. Subject M followed the same routine, but the examination was conducted between 6 and 7½ hours after entering the room. At 22 mm. depth the muscle temperature in B was 2.3° below that of the urine, and in M soon after the introduction of the needle the difference was 2.4° and half an hour later 3.9°, since the progressive cooling that has already been noted in the discussion of surface temperatures extended to the deeper tissues. Subject M was not in equilibrium and consequently his curve is somewhat distorted by progressive cooling; the distortion may be roughly estimated from the times noted and the values plotted at depths 22, 10 and 5 mm. respectively.

The data of November 23rd were obtained under almost identical conditions, except that M was examined first about 5 to 6 hours after entering the cooled room and 1½ to 2½ hours after the brief intermission for a light lunch; subject B was examined 6½ to 7½ hours after entering the room. The curve on M is almost identical with that obtained on January 12, but (with a somewhat shorter exposure) during immobilisation the fall in temperature of the deeper tissues was not quite so pronounced. The urine-muscle temperature difference was 3.55°. The curve in B obtained more than 2 hours later than on January 12 shows a greater fall in the deep muscle temperature, and a urine-muscle temperature difference of 2.63°, but the surface temperature was actually higher. Presumably the condition was nearer true equilibrium.

On January 7 subject B was warm, very uncomfortable and perspiring; M warm and rather uncomfortable; on the two cold days B was cold and comfortable, M cold and very uncomfortable.

On the two of the days plotted in figure 9 measurements of temperatures were made with thermocouple loops as an additional check; the comparison of the two sets of values is given in the subscription of figure 9 and has been referred to in relation to errors.

The deep temperatures in figure 9 represent muscle temperatures since the deep fascia can be demonstrated in these subjects at the approximate depths indicated in the figure. The position of this fascia may often be recognised by the resistance offered to the needle, by an aching or throbbing pain, accompanied by a nauseating sensation, and sometimes by a sudden increase in the temperature of the thermocouple after the fascia has been pierced; it is often visible in an x-ray photograph. In these experiments the criteria were not definite but the position of the fascia had been sufficiently established in these subjects.

The curves demonstrate the existence of a temperature difference between the muscles of the forearm and the deeper parts of the trunk. In a cold environment this difference is considerable; the progressive cooling of the surface, demonstrable in subject M, is also evident in the muscles. In a warm environment the difference is smaller; at 20 mm. depth in a very warm environment the muscle temperatures in both subjects were 0.25° to 0.30° below the urine temperatures, the difference being perhaps a little greater in subject M. Under comfortable environmental conditions this temperature difference was larger and was greater in M than in B.

The temperature gradient between the surface and the muscles were steeper when the environmental conditions were cold. Under warm condi-

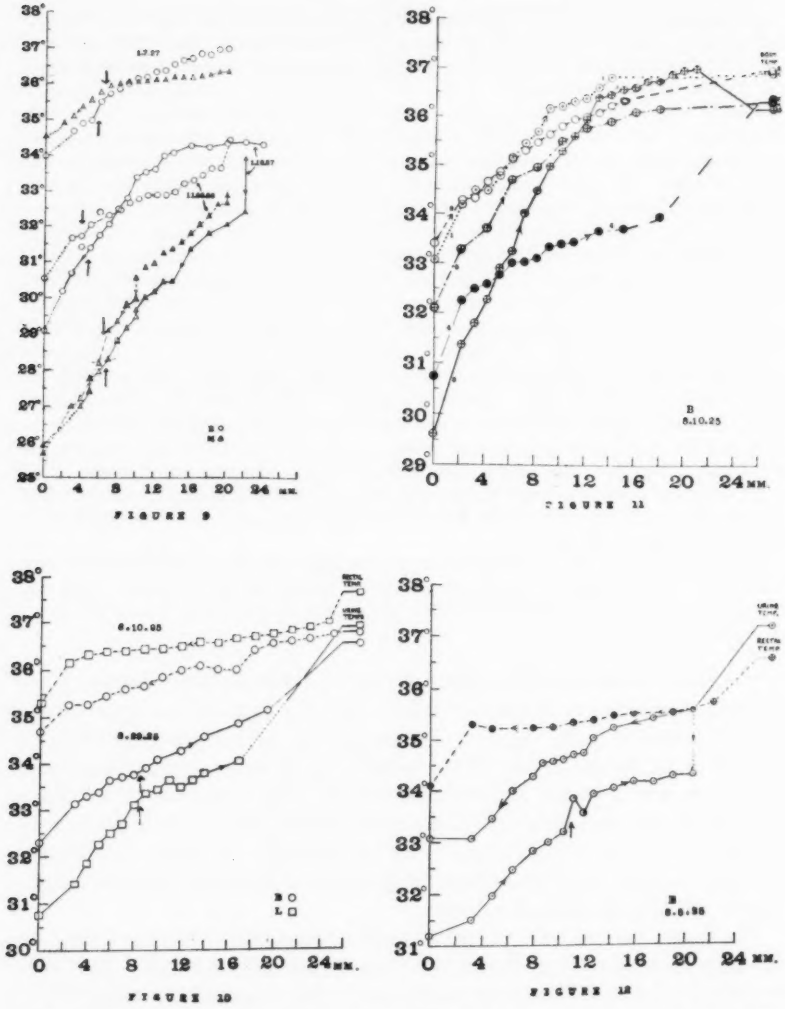

Fig. 9. Temperature gradients in the tissues of the forearm in subjects B and M under various environmental conditions. Ordinates: temperature. Abscissae: depths in mm. The upper two curves were obtained on January 7, 1927, under the following room conditions: D.B. 31.4 – 31.8°, W.B. 15.3°, D.K. 2.2, W.K. 15.8, E.T. 23.5°; the room conditions under which the two curves were obtained on November 23, 1926 (the inner curves of the lower group), were D.B. 18.5°, W.B. 7.1°, D.K. 6.8, W. K. 19.1, E.T. 12.8°, while the conditions for the other two curves (those of January 12, 1927) were D.B. 18.3 – 18.5°, W.B. 8.0°, D.K. 6.8, W.K. 19.1, and E.T. 12.8°. The following symbols have been adopted in this and succeeding figures: Subject B, forearm values obtained under all experimental conditions except after swimming ○, and after swimming ●; thigh, similarly under ordinary conditions ⊙ and after swimming ⊕: Subject M; forearm △: all other subjects (except Wi), fore-

arms under ordinary conditions □ and after bathing ■. The probable position of fascial planes is noted by vertical arrows: of these, those occurring about 4 to 9 mm. deep in the forearm or 12 to 14 mm. in the thigh, represent the deep fascia between the muscle and subcutaneous fat.

In the curve of subject M of date of November 23, 1926, the 0.2° fall of temperatures at depth of 20 mm. occurred within 9 minutes, the 0.6° fall at 10 mm. within 12 minutes; for the same subject of date of January 12, 1927, the 1.5° fall at 22 mm. depth occurred within 30 minutes, the 0.2° fall at 10 mm. within 5 minutes and the 0.3° fall at 5 mm. within 15 minutes.

Temperatures obtained with loop thermocouples as compared with corrected temperatures from needle thermocouples at same depths are as follows:

	DEPTH	LOOP THERMOCOUPLE	NEEDLE THERMOCOUPLE
	mm.	°C.	°C.
Subject B, January 7	3.5	34.8	34.8
Subject B, January 7	5.5	35.1	35.2
Subject B, January 12	4.2	30.8	31.1
Subject B, January 12	5.0	31.2	31.4
Subject M, January 7	5.2	35.5	35.6
Subject M, January 7	6.0	35.6	35.7
Subject M, January 12	3.5	27.3	26.9
Subject M, January 12	5.0	28.1	27.8

Fig. 10. Gradients observed in the forearm in subjects B and L on two different days; June 10, D.B. 29.1°, W.B. 21.1°, D.K. 2.3 and W.K. 12.4, E.T. 23.9°; August 29 D.B. 19.0°, W. B. 15.5°, D.K. 5.2, W.K. 16.3, E.T. 17.6°. The observations were made on June 10, B, 12:30 to 1:30 p.m., L, 3:40 to 4:05 p.m. and on August 29, L, 8:30 to 9:00 a.m. and B, 9:10 to 9:40 a.m. For further description see text.

Fig. 11. Gradients in forearm and thigh in subject B, both before and after bathing; August 10, D.B. 22.8°, W.B. 20.9°, D.K. 4.3, W.K. 14.2, E.T. 21.1°. Curve 1—Thigh before bathing ¾ to 1¼ hour after undressing. Curve 2—Arm before bathing 1¼ to 1¾ hour after undressing. Body temperatures from urine. Curve 3—Thigh 13 to 23 minutes after 20 minutes swim in water at 20.4°. Curve 4—Forearm 41 to 54 minutes after leaving water. Curve 5—Thigh 60 to 69 minutes after leaving water. Sensations: 1, neutral; 2, comfortable—slightly cool; 3, very cold with shivering; 4, comfortable; 5, comfortable. Between records 3 and 4 (20 to 33 minutes after leaving water), values were obtained during the withdrawal of the thigh needle at a time when shivering abruptly ceased and the temperature sensations were almost neutral. Body temperatures recorded after bathing were obtained by rectal thermocouple. The rectal temperature initially during record 3 was 36.16° and finally 36.24°; during withdrawal of the needle (not plotted) the rectal temperature returned to 36.16°. The only other noticeable change accompanying the greater comfort was a rise of the surface temperature to 31.0°, with a more gradual but otherwise similar gradient to 8.2 mm. depth. Beyond this depth, the gradient lay along the curve no. 3 with a slight rise of 0.1 to 0.2° superficial to the fascia and a fall of 0.1° within the muscle.

Fig. 12. Gradients in thigh in subject B, before (⊙) and after bathing (⊕) August 5, D.B. 23.7°, W.B. 20.7°, D.K. 4.1, W.K. 13.3, E.T. 21.25.° Lowest curve, introduction of needle, 20 to 50 minutes after exposure of thigh, rise in temperature at 20.8 mm. throughout period of 8 minutes due to inflammatory reaction. Middle curve; withdrawal of needle within subsequent 10 minutes. Upper curve; 65 to 75 minutes after 10 minutes swim in sea-water at 21.6°; subject during this interval "remarkably warm and comfortable."

tions the gradient was flatter in subject M than in B, especially in the muscular layers; this contrast was consistently observed.

The data on subject L are limited to two experimental days but are interesting since the contrast between subjects B and L is similar to that already noted between subject B and M, and the comparisons were made when the general outdoor conditions had varied instead of employing artificial room conditions. Subjects B and L were studied on the same experimental days; in Philadelphia after over a week of continued intense heat and on the northern sea coast on a cold morning (fig. 10).

The measurements made on subject L on both occasions and on subject B on the colder day were commenced about a quarter and completed about three-quarters of an hour after baring the arm. In spite of this the surface temperatures showed little variation except for a fall of 0.5° in the case of B on the colder day. The fascia was recognised on the colder day, when the needles were introduced gradually, but not on the warmer day where they were withdrawn by stages; in every case it was probably at about 8 mm. depth. The high rectal temperature recorded in subject L on the warm day is of no special significance, since the previous conditions were not controlled. On the warm day both subjects felt uncomfortably warm and subject B was definitely sweating; on the cold day subject B, while cool, felt quite comfortable, and subject L was uncomfortably cold. Subject L has resided under conditions similar to those of M.

On the warm day a third subject (J) was also examined, and a curve obtained located almost exactly midway between the other two curves up to a depth of 15 mm.; the curve reached 36.7° at 24.8 mm. depth; the urine temperature was 37.5°.

An analysis of the gradients of the superficial six millimeters. It is impossible to make an accurate analysis of the gradients in this area with vertical needles. A number of observations have been made with loop thermocouples (Bazett and McGlone, 1926b); it is difficult to introduce more than two in any experiment. However, from such data composite curves may be drawn. The validity of such curves depends on the absence of individual differences. The data are given in detail in table 3, and in table 4 those obtained on subjects B and M are grouped separately.

The apparent discrepancy in table 4 at depths 0.5 and 0.8 mm. can be accounted for since the mean environmental temperatures were not identical. There is therefore no evidence of any detectable individual variation in the dermis between these two subjects. In figure 8 these composite data are plotted on the basis of depth of thermocouple and are grouped according to environmental conditions. Since the circulatory condition varies with the temperature, inflammatory vascular dilatation has been induced for comparison. The data with inflammation are not grouped on a basis of environmental conditions.

The data given in table 3 for subject Br for an effective temperature of 14° were obtained during the subsidence of a hyperemic reaction which followed the introduction of the thermocouples; the three observations were made 20, 40 and 60 min-

utes after insertion. In a few instances, in three subjects, hyperemia was induced by burning lateral to the area under examination. Usually, histamine punctures (1/300 solution of ergamine phosphate) were laid down in a series of circumferential points 5 to 10 mm. distant, following the technic of Lewis and Grant (1924).

TABLE 3

Difference in temperature between surface and thermocouples of varying depths

SUBJECT	E. T.	SURFACE TEMPERATURE	DEPTH	TEMPERATURE DIFFERENCE	DEPTH	TEMPERATURE DIFFERENCE
	°C.	°C.	mm.	°C.	mm.	°C.
B	12.5	30.92	4.5	1.11	5.0	1.43
B	13.3	27.19	1.1	0.31	1.7	0.18
B	*13.3*	*34.14*	*1.1*	*0.42*	*1.7*	*0.24*
B	13.9	29.26	1.3	0.21	2.6	0.39
B	14.8	29.20	0.7	0.06	1.3	0.38
B	*14.8*	*32.41*	*0.7*	*0.41*	*1.3*	*0.54*
B	17.8	33.33	1.0	0.61	2.7	0.05
B	*17.8*	*35.69*	*1.0*	*0.61*	*2.7*	*0.00*
B	18.6	31.69	2.4	0.61		
B	21.1	32.98	0.5	0.24		
B	21.4	34.07	3.6	0.88	5.5	1.02
B	21.9	34.43	1.0	0.39	3.0	0.69
B	24.2	33.20	0.6	0.37	3.6	1.00
M	12.3	27.35	2.1	0.11		
M	*12.3*	*29.88*	*2.1*	*0.16*		
M	12.5	28.05	3.6	1.33	5.5	1.60
M	13.0	26.73	1.2	0.18	3.6	0.93
M*	15.5 to 17.4	30.25	0.9	0.30	1.7	0.13
*M**	*15.5 to 17.4*	*33.59*	*0.9*	*0.41*	*1.7*	*0.10*
M	17.9	32.30	0.9	0.62	2.0	0.57
M	*17.9*	*33.65*	*0.9*	*0.26*	*2.0*	*0.43*
M	20.8	33.80	2.0	0.72		
M	21.4	34.96	5.2	0.99	6.0	1.08
M	21.6	32.77	0.8	0.53		
M	21.7	34.11	0.7	0.23	1.9	0.16
M	23.6	34.32	0.7	0.29	2.6	0.59
Br.	*14.0*	*30.34*	*0.4*	*0.16*	*2.2*	*0.34*
Br.	*14.0*	*28.80*	*0.5*	*0.03*	*2.0*	*0.69*
Br.	14.0	27.51	0.5	0.07	2.9	0.31
Br.	14.6	31.07	1.2	0.36	2.2	0.83
Br.	*14.6*	*31.13*	*1.2*	*0.32*	*2.2*	*0.40*
Br.	16.7	31.16	0.9	0.23	2.0	0.37
Br.	*16.7*	*34.03*	*0.9*	*0.39*	*2.0*	*0.37*
K	21.6	33.77	0.7	0.16	1.6	0.47

The varying subjects are indicated by the letters and figures in italics indicate values obtained from areas showing an inflammatory hyperemia. The values marked with a star were obtained with differential thermocouples.

To exclude possible errors in measuring small temperatures differences differential thermocouples of the loop type were constructed with which the temperature difference between the surface and the point investigated could be directly measured by a single galvanometer. These couples were made from a single constantan wire, with the manganin elements soldered directly to the copper leads. The copper-manganin junctions were protected from irregular thermal changes by a single copper shield. The absence of parasitic currents was established by comparing the deflections after reversing the leads. Data so obtained are indicated in table 3 by asterisks.

The complicated character of the curves must be real and have an anatomical basis. The temperatures recorded by couples at a depth of 1.6 mm. were commonly close to those of the surface and lower than those at 1.0 mm. At depths beyond 4.0 mm. the composite curves of figure 9 are in complete agreement with the individual data of other experiments charted. Inflammatory hyperemia raises the temperature of the surface and produces gradients in the dermis similar to those that obtain in a warm environment. With a local hyperemia under cold conditions the surface temperature may

TABLE 4

Mean temperature gradients in degrees per millimeter at varying depths

SUBJECT	SURFACE TO DEPTH			
	0.5 to 0.8 mm.	0.9 to 1.3 mm.	3.3 to 4.5 mm.	5.0 to 6.0 mm.
B	0.38	0.33	0.26	0.23
M	0.48	0.33	0.31	0.22
Other subjects		0.28		

remain relatively low; in subject Br, after production of a histamine hyperemia, the surface temperature rose 1.59° but reached 31.13° only. It must be borne in mind that in a cold environment the deeper tissues have a low temperature.

Forearm gradients in other subjects. Considering subjects other than B and M it is impossible at present to decide whether differences observed in the character of the curves are due to the environmental conditions or to personal variations, since the number of normal curves obtained on any one subject has never exceeded three, and usually only a single series of observations has been made. In figure 13 some normal values are charted for subjects Wi and Y. The two subjects were examined on July 16 within a short interval after they had followed a similar routine for two hours. The curves should therefore be comparable, and their similarity is noticeable. Both subjects were thin, both were sensitive to cold, and both lived in the Great Lakes district. It will be noted that in Y the maximum temperature was reached at about 13 mm. depth, and that the temperature fell again at depths greater than this. This subject has a small forearm (18 cm. circumference) and probably when it was lying on a cold table, the cooling effect of the contact influenced the deeper tissues. The estimated temperatures at depths of 3.2 mm. in both subjects

exceeded the surface temperatures to an extent rarely found in subjects B or M under comfortable conditions. In subject Y (July 6, fig. 13, no. 5) the difference between a depth of 2.4 mm. and the surface was estimated as 2.5°; though the surface measurements were made three times in this experiment and always with a similar result, the surface temperature and the superficial gradient calculated from it must be regarded with suspicion. The lower level of the curve on this date as compared with the other may be explained as the result of a colder environment (effective temperature of the room 21.4° as compared with 22.2°).

The dermis in subject Y was thicker than that observed in other subjects (as evidenced during the needle introduction), and his skin was dry and rough. His reactions were unusual since he was not more sensitive to cold air conditions than others, but he was unable to withstand cold when bathing. It is, therefore, probable that individual differences in the dermal gradient may occur, although absent in subjects B and M. Subject Wi was somewhat similar to Y, but was not investigated to the same extent; no divergence from the normal thickness was noted in his skin.

Curves representing gradients in a female subject (H) are reproduced in figure 14; the two normal curves were obtained on one occasion during the introduction and withdrawal of the needle. With the exception of the previous winter and spring the subject had continuously resided in New England. The curve is similar to those obtained in male subjects in a similar comfortable environment. On withdrawal slight inflammation was observed and the gradient was somewhat modified. Two normal curves were obtained from temperature determinations on another New England female subject (O). These were similar to the general type; this subject withstood cold very well.

Gradients determined in another subject (Wa) are reproduced in figure 15. Since this subject always showed a very noticeable inflammatory reaction, they will be discussed under the general heading of effects of inflammation.

Tissue gradients in the thigh and the effect of the environment in subject B. The gradients existing on the inner side of thigh in subject B some two to three hours after entering a warm room are shown in figure 6B. Comparison with the forearm gradients in this same subject on June 10th plotted in figure 10, demonstrates that under warm conditions the surface and deep temperature in the two situations are similar. Any differences that may be distinguished could be due to chance anatomical variations, or to an incomplete establishment of equilibrium within the limited period of exposure to the warm conditions of the experiment illustrated in figure 6. The differences between the forearm and the thigh are much more evident under colder conditions. Values obtained on the same day on both arm and thigh are plotted in figure 11. (The other curves obtained on the same day after swimming will be discussed later.) The surface temperature on the thigh was the lower but beyond depths of 8 mm. the deep temperatures of the thigh were the higher; the gradient of the thigh was somewhat steeper than that of the forearm, though similar in character. The similarity is somewhat surprising. In the forearm the deep fascia offered resistance to the needle and could be recognized at about 7 mm. depth. In the thigh fibrous septa offered resistance and were recognized at depths of 7 and 9 mm., but the needle still remained freely movable in the subcutaneous fat and the true fascia was at a depth of between 13 and 14 mm. This difference in the thickness of the subcutaneous fat in this subject in the two situations was confirmed in all experiments made.

Other values for the forearm and thigh in this subject have already been published (Bazett and McGlone, 1926), the values being obtained on a day with an effective temperature 19.7° (dry bulb 21.7°, wet bulb 18.7°, dry kata 4.8, wet kata 16.2); the forearm was examined first (18 to 40 minutes after undressing and putting on a

bathing suit), and the thigh measurements were made later (45 to 70 minutes after undressing). On this day with a lower effective temperature the contrast between the forearm and thigh is similar to that demonstrated in figure 11, but is much more marked. Curves representing thigh gradients are also reproduced in figure 12. The lower of the two curves reproduced was obtained during the introduction of the needle, which caused considerable discomfort at its greatest depth with the immediate appearance of a large hyperemic ring round the entrance of the needle. The needle was left in position at the greatest depth for 7 minutes and during this time the deep temperature rose considerably as indicated in the figure. The higher of the two curves was obtained on withdrawal and the rise in surface temperature as the result of the inflammation is also evident. The complex curve obtained at the fascial level during introduction is probably due to some varying relationship of the needle to blood vessels.

Inflammation and its effect on gradients. Inflammation occurs around every puncture; usually the area is small and is covered by the rubber tip of the brass needle cover. It seems unlikely that an inflammation of this order, probably closely limited to the needle tract, can have much effect on the tissue temperatures, and there is evidence that the effect it produced was negligible. The surface temperature usually fell initially (owing to the heat withdrawn while the needle was attaining temperature equilibrium) and then rose to its original value, and if curves of the temperature gradients were plotted both for the introduction and withdrawal of the needle, a procedure which required 30 minutes, the two curves were often identical, except for slight variations due probably to errors in estimation of the depth. An example may be seen in the forearm curves of subject Y obtained on July 16 (see fig. 13, no. 2) where the surface temperature fell slightly during the 30 minutes of immobilisation; it was estimated initially as 33.69°, as 33.55° after 15 minutes and finally as 33.45°; it will be noticed that the curve obtained on withdrawal suggests that this cooling extended into the tissues, and that the whole temperature level of the forearm had fallen slightly.

In marked contrast were the values obtained, when considerable inflammation was observed. Such inflammation resulted in a hyperemic area occasionally exceeding 2 cm. in diameter. In subject Wa the hyperemia became evident when the point of the needle came into contact with the deep fascia (some 6 to 9 mm. deep in the forearm). Up to this depth readings of subcutaneous temperature could be made without this error being introduced. In the experiment of July 17 on subject Wa the surface temperature only rose 0.06° in the 20 minutes after the introduction of the needle; then the fascia was pierced and the surface temperature rose 1.06° in the next 15 minutes, although the hyperemia was less than that commonly observed in this subject. The induction of a hyperemia by needle puncture is accompanied by pain.

In the charts of figure 15 (subject Wa) the curve obtained on insertion on July 17, presumably shows the normal gradient existing in the subcutaneous tissue in this

subject before the onset of inflammation; and the curve on withdrawal demonstrates that the inflammatory rise of temperature extends throughout the whole of the subcutaneous tissue. It will be noted that the muscle layers show an absence of gradient both during introduction and withdrawal of the needle; since the inflammation commences as soon as the deep fascia is punctured it is not possible to say how far this is secondary to the hyperemia. It is difficult to believe that the temperature of the muscle is capable of any great and sudden change; the observations on August 28th were obtained as rapidly as possible after puncture of the deep fascia.

Another type of temperature change due to inflammation is illustrated in figure 14 (subject H). The upper curves show the entrance and withdrawal of a needle during a total period of 21 minutes; some tenderness was noted at 8 mm. depth (fascia) and again just beyond the deepest point charted. On withdrawal a slightly hyperemic area appeared, accompanied by a rise of the surface and the superficial subcutaneous temperatures, but by a fall of the deep subcutaneous temperature.

A third type of temperature change as the result of inflammation can be seen in figure 12, where the whole temperature level in subcutaneous tissue and sartorius muscle was raised nearly 1½ degrees, with little change in the character of the curve, except that the rise of temperature was greater nearer the surface than in the muscle layers. The usual tenderness was noted at the fascial level of about 13 mm. (thigh) and another point of extreme tenderness, causing the subject to sweat, was encountered at 20.8 mm. depth; the inflammatory reaction in this case appeared to be associated with the latter of these sensations.

The differences seen in the alteration of the curves by inflammation on different occasions may depend on variations in the point of greatest tissue irritation (compare last example quoted), or on the position of the thermocouple in the tissues, which in some cases might be close to a vein and in others close to an artery. The effect of inflammation on the gradients in the first two millimeters may be noted in figure 8 and table 3.

Temperatures in vessels. The temperatures existing in the peripheral blood vessels may to some extent explain the low temperatures in the muscles.

In dog 3 (table 1) a needle was introduced 7.7 mm. obliquely in the groin so that it lay in the wall of the femoral artery, and another needle was similarly introduced and lay close to the femoral vein; the temperature recorded by the former (after correction) was 38.09° and by the latter 37.41° when the rectal temperature was 38.55° and the surface temperature on the shaved groin 35.26°. The skin was divided, the exact position of the needles ascertained, and then one was introduced into the lumen of the artery for a length of 7 mm. and the wound covered. It then recorded a temperature of 37.98° (no correction considered necessary since it was in a stream of fluid). It is evident that the temperature within the femoral artery at the groin was about 0.5° below that of the rectum, though the room was very warm. The tissue close to the femoral vein had a temperature over a degree below that of the rectum. In the experiment on dog 4 (table 1) a needle was similarly introduced obliquely 15 mm. and was afterwards found to be lying between the femoral artery and femoral vein; it gave a corrected value of 36.96° when the rectal temperature was 37.90°. Two needles were then introduced into the artery and into the femoral vein after exposure and the wound was closed with stitches; the temperatures then recorded were femoral artery (no correction) 36.55°, femoral vein (no correction) 36.19° and rectal

37.19°; the surface temperature was 32.39°. The lower temperatures in the femoral vein and in other tissues in close proximity to the artery are sufficient to account for the difference in temperature between the femoral artery and the rectum. In contrast another animal, at a room temperature of 33.0° dry bulb and with considerable humidity, had a rectal temperature of 39.65°, a temperature in the femoral artery of 39.34° and in the femoral vein 39.02°, when the surface temperature was 36.16°.

Two attempts have been made to puncture arteries in subject B with thermocouple needles, but it was not possible to determine whether the lumen was entered, since the needles do not allow hemorrhage from a deep location. Both attempts were made when the room temperature was 18.45° D.B. (November 23, 1926, for conditions see fig. 9) when the subject had been exposed to the environmental conditions for about 8 hours. A needle was inserted obliquely at the elbow in the direction of the artery to a depth of 15 mm.; the temperature observed was 34.4°; on a reintroduction of the needle to the same depth a sensation was noted similar to that commonly caused by arterial puncture and the temperature observed (corrected) was 35.9°; the urine temperature was 36.8°. (A previous puncture of the median cephalic vein with the thermocouple gave an uncorrected reading of 34.9°; the venous blood may have been of muscular origin, since the cutaneous veins were strongly constricted.) Immediately after this the thermocouple was introduced vertically in the region of the femoral canal (directly after the skin was bared) to a depth of 20 mm., the needle passing one definite fascial resistance; the temperature after correction was 36.6°; the needle was further introduced to a depth of 25 mm. and passed a second resistant layer; the temperature after correction was 36.8°, and the urine temperature as previously stated was also 36.8°. No sensation such as that associated with the puncture of smaller arteries was noted, but if the vessel was pierced no correction ought to be applied, in which case the higher temperature should be regarded as 36.7°.

On other occasions veins in the forearm have been punctured with the following results; in a room at 26.3° D.B. the temperature in a vein at the base of the left index finger was 35.1° and that in the median cephalic 34.6°, thus demonstrating that cooling may occur along the superficial veins. On a cooler day (D.B. 18.4°, E.T. 14.4°) the temperature in the median cephalic was 34.0°. Another subject on the same occasion gave a reading of 32.8°, and in this case the superficial cutaneous veins were less constricted than in subject B. No corrections have been considered justifiable in the above readings.

EFFECTS OF SEA BATHING. The routine described below was followed.

The subject entered the water and swam throughout the period with short intervals for diving. All experiments were made at Woods Hole on the southern side of Cape Cod. After bathing, the subjects, with two exceptions, ran to the laboratory, a distance of not more than 100 yards. The male subjects removed their bathing suits, were allowed a few seconds to mop themselves with a towel, and remained unclothed during the period of the temperature determinations. Rectal temperatures were taken by a thermocouple. The female subjects did not remove their wet bathing dress, but dried the forearm. Body temperatures were estimated by urine temperature measurements made just before the bath and after completion of all the other measurements (some 30 to 60 minutes later).

Changes of rectal and surface temperature. The changes in rectal and surface temperatures, following a swim in three experiments on subject B and one on subject Y are charted in figure 16. The body temperatures

observed before the bath are in every case values determined from the urine, but such values compare well with rectal temperatures determined simultaneously with thermocouples; in the experiment on subject Y the final rectal temperature (thermojunction at 10 cm. depth) was 36.35° and a urine temperature taken at this time gave a value of 36.4°. The body temperature showed a marked fall in spite of the muscular activity; shortly after leaving the water a rise occurred in subject B, but none in Y, though at this time Y was shivering more violently than B. In subject B, after the rise of rectal temperature the sensation of cold disappeared, and the shivering ceased; then the rectal temperature fell unaccompanied by any sensation of cold. Later the rectal temperature variations were slight and irregular. In general, therefore, there is a profound and lasting fall of deep body temperature produced by the swimming. Subject Y maintained an almost constant low temperature level for over an hour after the swim, when shivering had ceased without rise in rectal temperature.

Since the principal object of the experiments was the determination of tissue gradients, the records for surface and rectal temperatures are incomplete. The surface temperatures first determined after bathing in subject B were those on the thigh; forearm determinations either followed those on the thigh or were made in an interval between successive thigh measurements. All values for subject Y were obtained on the forearm. In two experiments, after a period of 80 minutes, surface temperatures were recorded above the values found before the bath (B thigh on August 5th, Y forearm), and on both occasions the subjects at this time experienced a warm glow. Emphasis cannot be placed on this apparent correlation of sensation with surface temperature. In spite of the fact that subject Y felt very cold and was unable to remain immersed longer than 9 minutes, though the day was above the average warmth, no special character is detectable in his curves, except that the rectal temperature showed no rise during the period of observations.

The question arose whether the measurements of surface temperatures and gradients in the thigh and forearm gave an accurate impression of the changes in the body as a whole. Surface temperature measurements following swimming were made, using the positions designated by Benedict (1925). Subject H was used and all the observations were kindly made for us by Dr. Elizabeth Crofts, to whom we are much indebted. The subject lay without clothes on a table for preliminary observations before the swim, and measurements were taken twice afterwards, during the first half hour, and during the following 20 minutes. As the surface thermocouple used was of our oldest pattern with rather heavy wires, equilibrium was attained rather slowly, so that the whole series of observations occupied some time, and different parts of the body can only be compared approximately.

The baths were of 26 minutes and 30 minutes duration in water about 21°. The urine temperature fell 1.2° and 1.0°, respectively, during the swim and the subsequent 50 minutes of exposure without clothes. The temperature was read first in position 14 (over the liver) and showed an initial fall of 3.2° and 2.3° in the two experiments, but after ½ hour it had regained 1.4° and 0.3°. Readings were made on the feet some 25 and 45 minutes after leaving the water, and showed the greatest change, position 24 L (bottom of left foot) showing a fall of 6.0° and 3.9° in the initial readings on the two occasions and a recovery of 2.0° on the first occasion and in the second experiment no change at the time of the second reading. The extreme differ-

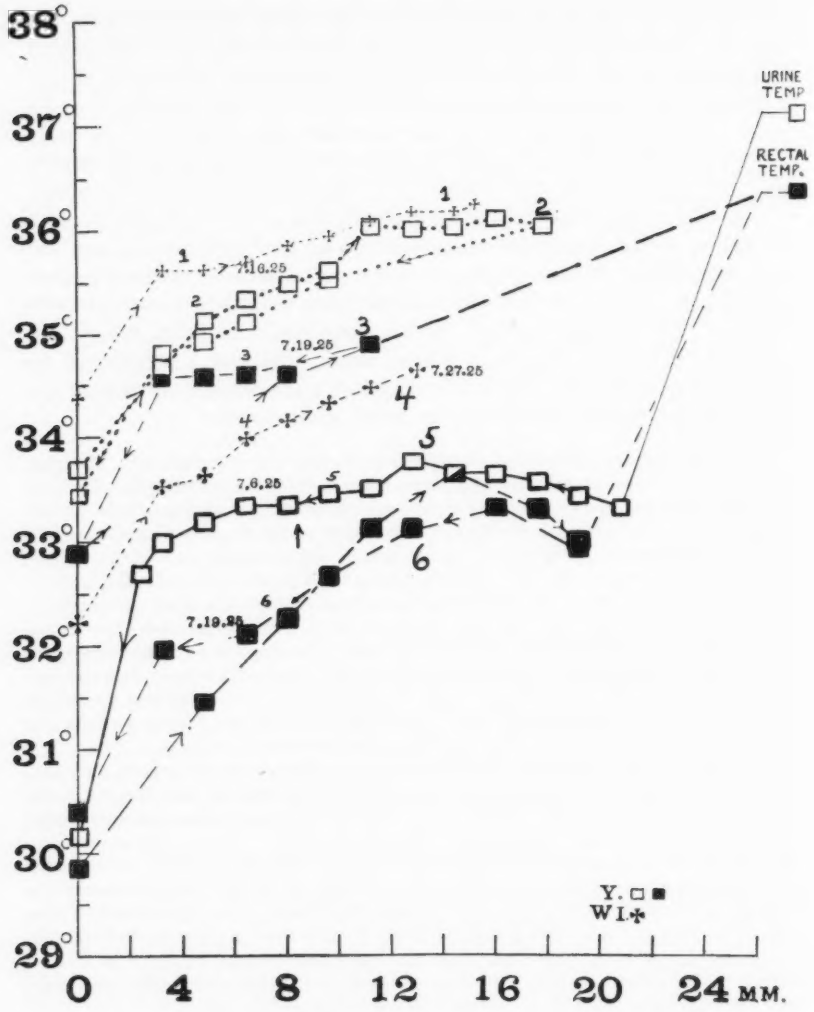

Fig. 13. Gradients in forearm of two subjects (Y and Wi); July 6, D.B. 23.5°, W.B. 20.5°, D.K. 4.0, W.K. 11.3, E.T. 21.4°. July 16, D.B. 24.5°, W.B. 21.5°, D.K. 4.0, W.K. 11.4, E.T. 22.2°. July 19, D.B. 25.3°, W.B. 19.3°, D.K. 3.9, W.K. 11.5, E.T. 21.7°. July 27, D.B. 23.0°, W.B. 20.3°, D.K. 4.7, W.K. 12.8, E.T. 20.8°. July 6, subject Y, (□ no. 5), 15 to 45 minutes after exposure of arm. July 16, curves of subject Y, (□ no. 2), introduction and withdrawal of thermocouple needle during period of 15 to 45 minutes after exposure. Curve of subject Wi, (+ no. 1) from data obtained under similar conditions; urine temperatures, subject Y,

ence between any two points (over liver point 14, to foot) were before bathing 3.7° and 6.8°, during the first ½ hour after bathing 4.3° and 8.3° and during the second ½ hour 6.2° and 7.3°.

Bathing produces, therefore, effects similar to those described by Benedict on exposure to cold air. As the result of the bathing the mean fall of temperature of the 20 positions examined was 3.51° for the first ½ hour, with a mean rise of 1.31° in the next ½ hour. On these occasions the thigh surface had fallen 3.4° when examined during the first ½ hour and had risen 1.15° by the time of the second reading, while the forearm fell 2.5° and had risen 0.15° only. The thigh, therefore, gives values close to those of the general mean.

Changes in tissue gradients. The gradient changes in the tissues are such as might be expected from the surface and deep temperature changes. Examples of the gradients observed in the forearm and thigh may be seen in figures 11 to 15 and in the figure previously published (Bazett and McGlone, 1926a).

Forearm. Immediately after the baths both the surface and muscle temperatures in the forearm were below normal; the gradients between the surface and the muscles and the contrast between the muscle temperature and that of the rectum were increased. The entire forearm had lost heat. A little later, the deep muscle temperatures were found to be falling while those of the surface and more superficial subcutaneous tissue were rising. The gradient, therefore, changes in slope pivoting at a depth of 6 to 8 mm., at no great distance from the position of the deep fascia. Accompanying the fall of muscle temperature with a decrease in the gradient in the forearm, subjects H, Wa and Y, invariably had an increase in shivering with sensation of cold.

Records were also made on a female subject (O) who was accustomed to bathing in the colder water north of Cape Cod, and who noticed no cooling effect when swimming in water of 20 to 21°C. This subject bathed in water at about 21.5° for one hour and was examined within a period of 4 to 26 minutes after leaving it, and while still in a damp bathing dress. The surface temperature of the forearm was 31.05°

36.95°, subject Wi, 37.15°. July 19—lower two curves subject Y, (■ no. 6) obtained after swimming in sea-water at 21.5° for 9 minutes. Before the bath, surface temperature was 32.3°, urine temperature 36.8°. Needle introduced to 19.2 mm. and then withdrawn. First temperature determination 8 minutes, at maximal depth 18 minutes, final measurement 24 minutes after leaving water. Subject very cold, shivering increasing in intensity during examination. Rectal temperature constant. Third curve (■ no. 3), subject dressed and measurements made 54 to 60 minutes after leaving water. At end of period of observation the rectal temperature was 36.35° identical with that recorded immediately after bathing. Subject "warm". July 27, subject Wi (+ no. 4.), urine temperature before bathing 36.8°, after 37.0°. Swimming 15 minutes in water about 20°. Observations were made on subject dressed, 30 to 37 minutes after leaving water. During the following 8 minutes the needle was withdrawn, and temperature values (not charted) indicated falling subcutaneous temperatures (−0.2 to −0.3°). Subject felt very cold, his skin was pale and somewhat blue.

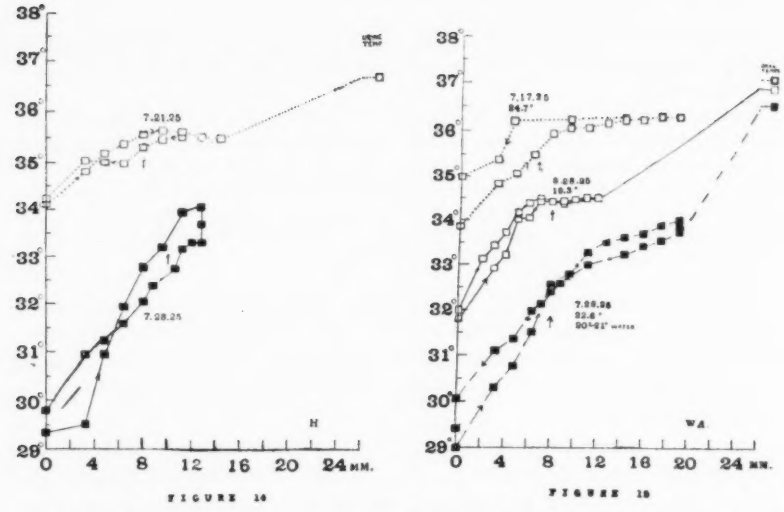

FIGURE 14 FIGURE 15

Fig. 14. Gradients in forearm of a female subject (H). Data obtained on bare forearm in usual dress indicated by open symbols (□), in wet bathing suit after swim by closed symbols (■). July 21, D.B. 24.6°, W.B. 20.5°, D.K. 4.0, W.K. 11.6, E.T. 21.6.° July 28, D.B. 22.6°, W.B. 19.5°, D.K. 4.4, W.K. 13.6, E.T. 20.5°. July 21, observations made 20 to 41 minutes after exposure of arm. Slight hyperemia during withdrawal of needle. July 28, observations after a 25 minute swim in water at 20 to 21°. Urine temperature before bath 37.2, at end of all observations 37.1°. The first observations were made 10 minutes after bathing; a depth of 12.8 mm. was reached 16 minutes later; the fall at this depth occurred during 8 minutes and needle was withdrawn over a period of 25 to 32 minutes after leaving water. Subject cool, but comfortable at first; during the withdrawal, colder and shivering slightly.

Fig. 15. Gradients in bare forearm of a female subject (Wa), in usual dress (□), and after bathing (■). July 17, D.B. 24.7°, W. B. 21.8°, D.K. 3.5, W.K. 10.9, E.T. 22.5°; July 28, D.B. 22.6°, W.B. 19.5°, D.K. 4.4, W.K. 13.6, E.T. 20.3°; August 28, D.B. 19.3°, W.B. 13.5°, D.K. 6.5, W.K. 21.6, E.T. 15.9°; July 17, the thermocouple needle was introduced 5 minutes after baring of arm, and observations began 10 minutes later; no inflammation was noted until contact with the deep fascia 20 minutes after introduction of needle. Maximum depth reached 32 minutes and withdrawal completed 40 minutes after exposure. Hyperemic area 2 to 3 cm. diameter. August 28, the needle inserted 5 minutes after exposure of arm; observations began 11 minutes; fascia reached 18 minutes later and all temperature measurements up to 12 mm. made during the next 5 minutes; the remaining observations were completed 41 minutes after exposure of arm. Increasing hyperemia noticed during withdrawal; the ultimate diameter of hyperemia was 2 cm. Subject observed cold sensations, but these were not referred to forearm. July 28, observations after swimming 30 minutes in water at 20 to 21°. Oral temperature before bathing 37.1°, at end of all observations 36.6°. No inflammation observed. Observations during insertion of needle 8 to 22 minutes after leaving water; fall of temperature at maximum depth occurred in 2 minutse' interval, and observations completed 32 minutes after swimming. Subject did not feel cold on entering room but the sensations of cold increased throughout period of examination. Gooseflesh on forearm occurred intermittently. Subject blue with cold and shivering slightly during period of withdrawal of needle.

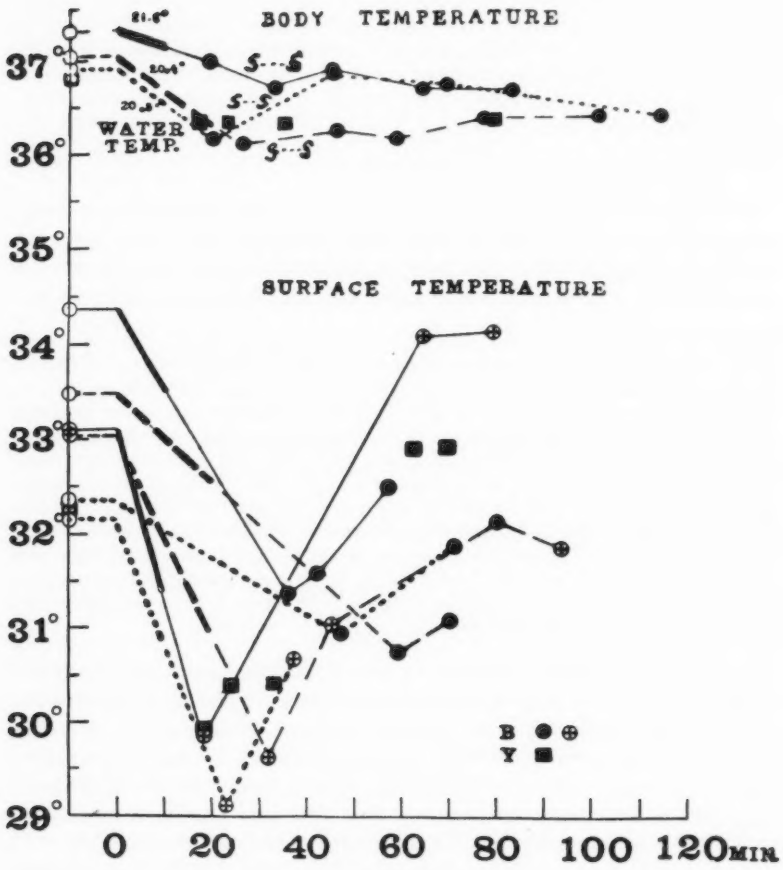

Fig. 16. Deep body temperatures and surface temperatures of forearm and thigh, before and after bathing in sea-water, plotted as ordinates, against time in minutes from entrance into the water as abscissae. Symbols as indicated in figure 9. Values for subject Y are not connected by lines. For subject B the data of experiment detailed in figure 11, indicated by dashes (----------); those of experiment detailed in figure 12 by continuous line (————); and those of experiment previously reported (Bazett and McGlone, 1926a) by dots (.....) for environmental conditions, p. 435. The period of exposure to the water indicated by the increased width of the line.

and rose to 32.4° within 8 minutes and had reached 33.45° within 17 minutes. On control days surface temperatures of 32.6° and 33.6° were obtained. On introduction of the needle there was no departure from a normal curve at a normal temperature level, except for a slight increase in the gradient near the surface corresponding with the slight fall of surface temperature. On withdrawal of the needle when the surface had regained its normal temperature, the gradient was also normal. The urine temperature fell from 37.5° before bathing (when the subject was uncomfortably warm from exercise) to a level of 36.95° at the end of the observations. No sensations of either cold or warmth were noted. The subject had a subcutaneous tissue of moderate thickness; the deep fascia was about 9 mm. below the surface.

Three-quarters of an hour after bathing, the gradients in the forearm of subject B were little different from those obtained under ordinary room conditions, though the levels were usually subnormal; the subject was comfortable. At a similar period subjects Y and Wi reacted differently.

Subject Y when dressed was reëxamined one hour after leaving the water. His rectal temperature was identical with that recorded at the earlier period directly after the bath; the surface had risen to a level between those recorded on the two control days and somewhat higher than that recorded on this day just before the bath. The gradient between the surface and 3.2 mm. depth was similar to his normal gradient, but at greater depths the gradient was subnormal. In spite of the low rectal temperature he described his sensations as "a warm glow" and considered himself as being warmer than prior to entering the water. Subject Wi (fig. 13, no. 4) was examined 30 to 45 minutes after leaving the water, when he had dressed; he complained greatly of cold, and his skin was pale and slightly cyanotic, though his urine temperature showed a rise of 0.2° (36.8° to 37.0°) when compared with that obtained immediately before the bath. The gradient in the forearm was steeper than normal and at a lower level than that recorded on the control day.

Thigh. The changes observed in the thigh gradients were similar to those in the forearm with the following exceptions. The heat production in the large muscular mass of the thigh during swimming and with shivering resulted in a rise rather than a fall of temperature in the sartorious muscle (figs. 11, 12 and that previously published) even though the rectal temperature had fallen. In consequence, after bathing, muscle temperatures considerably above those of the rectum were sometimes recorded (fig. 11). The muscle temperature fell while that of the skin and subcutaneous tissue rose as in the forearm, but the pivoting occurred at a depth of some 12 to 14 mm., with the exception of a single observation where the depth was about 8 mm. Therefore, the pivoting in the thigh, as in the arm, is commonly noted at about the depth of the deep fascia. In subject B the thigh gradients were investigated as soon as possible after leaving the water, and the forearm was only examined some $\frac{3}{4}$ hour later. On two occasions, however, (figs. 11 and 12) the thigh gradients were reinvestigated after the completion of the forearm examination (58 to 65 and 58 to 72 min. respectively after leaving the water). In the experiment plotted in figure 11, the later curve obtained was of normal type but at a somewhat subnormal level. The

sensation experienced was "comfortable throughout the period of observation but no definite warm glow;" on the other occasion the surface temperature was above normal and the gradient beyond 3 mm. depth, much flatter than that recorded on any other experiment in the thigh. The sensations on this day were different to those on any other day; a warm glow was noted 5 minutes after leaving the water and this warm glow continued, even though after 17 minutes there was a slight tendency to shiver (contrast marked shivering of other experiments) while at the time the thigh records were made, the subject noted "still feeling remarkably warm and comfortable."

Discussion. The tissues examined may be regarded as probably having the following thicknesses. Muchow (Tab. Biol., 1925) states that the thickness of the epidermis of the forearm and thigh varies between 0.04 over and 0.15 mm. between the papillae; the dermis and epidermis together may vary considerably but are likely in these regions to have a mean value (according to Muchow) of 1.8 to 2.4 mm. Some evidence is available as to the total thickness of the dermis and subcutaneous fat in the forearm of the subjects used. If the needle was withdrawn until the point lay 2.0 to 2.3 mm. below the skin surface, it could be reintroduced without meeting any resistance other than that afforded by the fat, except in subject Y as noted, so that the thickness of the skin of the other subjects was not greater than the figures given above. The dermal subcutaneous boundary could sometimes be distinguished in x-ray photograph; in subjects B and M a skin thickness of 2 mm. was thus indicated. The normal thickness of subcutaneous fat in average subjects is given by Muchow as 4 to 9 mm; in our subjects with the forearm flexed (to about 90°) the fascia could be recognized at depths of about 7 mm. in B, 8 mm. in M, 7 to 8 mm. in Y and L, 8 to 10 in H and 9 to 11 in O. A variation of one to two millimeters was noticed in successive experiments in the same subject. This has been correlated with the degree of flexion of the forearm; when the forearm is fully extended, the depth of the fascia is reduced to 3 or 4 mm. in B and to 6 mm. in M. The fascia can often be identified in x-ray photographs, and the values of the depth given for subjects B and M have been confirmed.

In the thigh the subcutaneous fat was thicker; in subject B the fascia was recognized at depths of about 13 to 14 mm., though other septa were often encountered at depths of 7 to 9 mm.

If the *general character of the gradients* in the skin, subcutaneous tissue and muscle be compared on a basis of warm days (E.T. 17° or greater) and cold days (E.T. less than 17°), on warm days the curves exhibit a convexity upward, while on the colder days the convexity is downward. If these gradients be more carefully analyzed, as in the composite curves of figure 8, where the temperature differences are plotted without regard to temperature level, the contrast between these two types is seen to depend

on the relative insulating value of the dermis and subcutaneous tissue. In warm conditions the insulating value of the fat is much less than that of the dermis, while the reverse holds true for cold.

The diminished gradients in the most superficial layers of the skin on the colder days were not anticipated. A decreased temperature gradient must imply a decrease in heat loss or an increased thermal conductivity. If, under warm conditions, the exposed parts of the limbs be used as a radiator system assisted by evaporation, an actual diminution of heat loss on the cold days is possible from this local exposed area; in a cold air environment with little evaporation the main heat loss may occur from other parts of the body.

In the *dermal area* the maximal temperature is usually at a depth of about 1 mm. The rapid rise in temperature between the surface and this maximal point does not appear to depend principally on the physical properties of the epidermis, since the gradient is steeper between the levels of 0.5 and 1.0 mm. than in the more superficial layers of dermis and epidermis. Deep to 1 mm. the temperatures are lower, the lowest level appearing to be at a depth of about 1.6 mm. Such a fall of temperature could be explained if the gradients depend on the anatomical distribution of the vessels, and if at this level there is a group of veins carrying blood which has previously been cooled in superficial capillaries. The arteriolar blood in such an area should have a relatively high temperature, but, if these vessels are few in number, the temperature of the thermocouples would only occasionally be affected, and then by chance. Spalteholz (1893) described four venous networks, one immediately below the papillae and associated with arterial vessels, with which the capillary loops are connected, a second deeper and superficial to the subpapillary arterial network, which then lies between the second and a third deeper venous network, the last being at the boundary of the middle and lower thirds of the cutis; at the boundary of the dermis and subcutaneous tissue lie the cutaneous arterial network and an associated fourth venous plexus. The irregularity of the values given in table 3 seems to depend on the position of the thermocouple relative to these vessels, and to indicate the presence of a subpapillary arterial network at a depth of about 1.0 mm., of a venous net at about 1.6 mm. and probably of a more superficial venous net at a depth of 0.5 to 0.7 mm. In the sole of the foot, with a skin of total thickness of 1.6 mm., the depths of the second venous net, subpapillary arterial net and third venous net were 0.7, 0.8 and 1.25 mm. respectively (Spalteholz). In our own experiments the living skin had a thickness of 2.0 mm., so that the results described appear to be due to these three vascular networks, and the anomalous reverse thermal gradient receives a simple explanation as the result of the return of cool venous blood. The differences observed between the gradients in the dermis on warm and cool days depend mainly on variations in vascular dilatation, as is indicated

by the gradients observed after the production of an inflammatory hyperemia. The apparent shift of the point of maximum temperature in the dermis to a deeper position on the cooler days cannot be considered significant, unless supported by much more numerous data.

The gradients in the subcutaneous fat are taken in these experiments as the gradients existing between depths of 2 to 6 or 9 mm. in the forearm, and from 2 to 12, 13 or 14 mm. in the thigh. Spalteholz (1893) described septa dividing the fatty layers into lobules, with a more definite septum separating the superficial from the deeper fat; the superficial layers receive their blood supply mainly from retrograde vessels passing centrally from the rete arteriosum cutaneum (at the junction of dermis and fat), while the deeper fat receives most of its blood supply from vessels piercing the deep fascia.

The high thermal conductivity indicated by the low gradient in the fat of the forearm under warm conditions may be explained by the special arrangement of its blood vessels. If vasoconstriction with cold and vasodilatation with warmth particularly affects the retrograde vessels, the observed variations in gradient might be anticipated. There is in the forearm usually little thermal gradient in the deeper fat close to the fascia except when the conditions are uncomfortably cool. Retrograde vessels in this area are described, but are few in number.

Gradients in the muscles. There is usually a steep thermal gradient across the deep fascia, but beyond this point it is greatly diminished. In the forearm at depths of 14 or 15 mm. an intermuscular septum can often be recognized. At this depth the temperature is not infrequently lower, so that minor variations are probably determined by the anatomical distribution of the vessels.

The temperatures in the muscles of the limbs at rest are considerably lower than the rectal temperature, and in a cold environment the difference is exaggerated.

Temperatures in the vessels. The few data reported confirm those of Claude Bernard (1876) and demonstrate that a considerable cooling of the blood may occur while it is still within the peripheral arteries, if the blood in the venae comites is returning at a low temperature. The cooling of the blood within the arteries affords a ready explanation for the low muscle temperatures. The blood in the superficial veins of the forearm has a temperature similar to that of the skin surface; if the cutaneous vessels be constricted so that blood of this origin constitutes a smaller proportion of the total content of the median cephalic vein the temperature in this vessel may be somewhat higher.

Mathematical treatment. It is difficult to treat mathematically such irregular curves as those described. Attempts have been made to find some empirical method of expressing their general slope. If the logarithms of

the temperature differences between the surface and at a point at any depth, be plotted against the logarithms of the depth, approximate straight lines can usually be drawn through the data, though in most cases two such straight lines must be used, which commonly form an angle at a position corresponding with that of the deep fascia. It is possible that the comparison of individuals from the consideration of values so plotted may eventually prove significant.

General sensations of temperature have been noted. Sensations of warmth may be accompanied by a decreased or sensations of cold by an increased rectal temperature, as has been previously well recognised. A sensation of cold may change to one of warmth without a change of rectal temperature in either direction. Changes in deep body temperature are not, therefore, the essential cause of such sensations, if an adequate evaluation of these changes is supplied by rectal or urine temperature determinations. Sensations of cold usually accompany a falling surface temperature, but such sensations can be very intense even when the surface temperature is rapidly rising—e.g., after sea bathing. There is no obvious correlation between such sensations and the direction of change of surface temperature. In the conditions investigated when rapid changes in sensation occurred (e.g., after swimming) there was always some layer of the tissues investigated in which the temperature was falling when the subject was feeling cold, or was rising when the subject was feeling warm. No correlation was noted between such sensations and the changes in any particular locality or depth, though it is difficult to exclude such a possibility.

Consequently, attention has been directed to the relationship of the spatial distribution of temperature to the sensations experienced. Sensations of cold have always been accompanied by a steep thermal gradient in the subcutaneous tissues, but in the forearm where dermal gradients have been measured, these have been less steep than usual. Comfortable conditions when temperature sensations are not perceived are accompanied by a moderately steep gradient in the more superficial parts of the fat, and usually by a smaller gradient (appearing in the curves almost as a plateau) one to three millimeters immediately superficial to the deep fascia. The gradient across the epidermis and superficial part of the dermis in the forearm is probably steeper than that observed in cold conditions. Sensations of warmth have been associated with a low gradient in the superficial part of the subcutaneous tissue; the gradients in the deeper layers of the fat are similar to those accompanying comfortable conditions. The gradient in the superficial part of the dermis are steep and greater than those in the fat. A definite thermal gradient may be constantly present in the superficial muscle layers in some subjects; these gradients are exaggerated when the subject feels cold.

It would be possible to attribute the sensations of general warmth to end

organs sensitive to an uncommonly low thermal gradient in the superficial layers of the fat, and those of cold to others sensitive to the presence of an uncommonly steep gradient in the deeper part of the fat, but it is equally possible that the gradients observed in the subcutaneous tissue are dependent on vasomotor changes secondary to such sensations. The two conditions appear to be related, but it is impossible to decide which may be the causal agent. The data obtained on the temperature changes observable in the dermis during stimulation of cold and warm spots (Bazett and McGlone, 1926b) will be reported in detail later.

SUMMARY

1. A needle thermocouple is described, and in addition others consisting of light manganin and constantan wire of low thermal conductivity and capacity. With these, measurements of the temperatures existing in the forearm, thigh and vessels of human subjects under varying environmental conditions have been made.

2. It has been demonstrated that the needle thermocouples have a systematic error; an empirical formula has been developed allowing an approximate correction to be made.

3. In the course of these investigations a number of surface temperature measurements on the forearm have been made on two subjects with somewhat different thermal reactions. The relation of the temperatures observed to the "effective temperature index" (of Yagloglou and his co-workers) is not identical in the two subjects, especially at the lower temperatures.

4. Examples are given of the thermal gradients observed in a number of subjects under varying environmental conditions. The temperatures existing in the muscles of the forearm close to the bones were under normal conditions commonly 1 to 2 degrees below the rectal temperature.

5. The gradients observed for the same environmental conditions varied in different subjects, but the same gradient was usually noted in any one subject, if the conditions were at all comparable. Inflammatory reactions were usually noted only in a limited area adjacent to the needle, and have been considered as only slightly modifying the thermal gradients. Occasionally an inflammatory hyperemia extending over one or two centimeters was noted; in such cases the surface temperature was raised, but the deeper temperatures might be either increased or decreased as the result of the inflammation.

6. Complicated temperature gradients in the dermis are described, and their relations to the anatomical descriptions of Spaltcholz are discussed.

7. Data have been obtained on the changes observable in deep body temperature, surface temperature and spatial thermal gradients as the result of swimming in cool sea-water.

8. Warm conditions, accompanied by general sensations of warmth were associated with thermal gradients of a low order in both the superficial and deeper portions of the subcutaneous fat and with a gradient in the most superficial part of the skin (1 mm.) of the forearm, which was steeper than at any other depth. Some subjects showed a slight gradient in the superficial layers of the muscle; more commonly this was absent.

Comfortable conditions, when general temperature sensations were not perceived, were associated with a moderately steep gradient in the superficial part of the subcutaneous fat, a very low gradient in the deepest part of the fat. A gradient in the superficial layers of the muscle might or might not be present.

Cold conditions accompanied by general sensations of cold, were associated with steep thermal gradients in both the superficial and deeper parts of the fat, and the gradient in the most superficial part of the skin of the forearm was below the average when direct measurements of this gradient were made, and less than that observed in the subcutaneous tissue. Under these circumstances a considerable gradient in the superficial layers of the muscle was the rule.

We take this opportunity of expressing our thanks to Dr. W. P. White, to Dr. F. G. Benedict and to Dr. H. C. Richards for useful criticism and advice on technical details, to the Ella Sachs Plotz Foundation which very kindly gave a grant towards the expenses of this research, as well as to students who have acted as subjects. We also desire to acknowledge the coöperation of Drs. R. J. Brocklehurst and E. H. Brunquist in a few of the experiments.

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THE TEMPERATURE OF THE AIR IN CONTACT WITH THE SKIN

B. McGLONE AND H. C. BAZETT

From the Department of Physiology, University of Pennsylvania

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It has been well recognised that a special layer of warm air surrounds the body beneath the clothing, and measurements of the temperatures of the clothed portions of the skin have been reported by Benedict, Miles and Johnson (1919), Benedict (1925a, b) and Loewy and Dorno (1925). The warm air layers beneath the clothes have been termed the "private climate." Loewy and Dorno (1925) found that the air beneath the clothes was commonly only some 4 or 5 degrees below that of the adjacent skin surface, and the difference did not exceed 7 degrees under the coldest conditions in which they worked. The same workers report two measurements of the temperature of air in the neighborhood of the bare forearm; they report the following observations:

SURFACE	1 MM. DISTANT	3 MM. DISTANT
30.2°	28.9°	28.5°
29.9°	28.7°	28.5°

No other attempts appear to have been made to determine the temperature gradients existing in the air layers in contact with bare skin.

TECHNIQUE. Thermocouples have been brought in contact with the skin surface of the bare extensor aspect of the forearm, and removed by ½ mm. steps to a distance of 5 to 6 mm. and then returned by similar steps to their original position until contact with the surface was reëstablished; temperature readings were taken throughout. Determinations were made under varying environmental conditions. The forearm was exposed bare to the experimental air conditions for at least one and usually several hours before the observations were made. An area some 2 to 3 cm. in diameter was shaved and observations were made on both shaved and unshaved areas. Only two subjects have been employed, namely, B and M, of whom descriptions have been given in the previous paper. The forearm was supported in an arm rest of the Mosso ergograph pattern.

The thermocouples employed were of the light wire (0.08 mm. diam.)

pattern of manganin and constantan described in the previous paper. This type of thermocouple, having a low heat capacity, is capable of rapid adjustment to altered air conditions.

The thermocouple had a resistance of about 120 ohms. The manganin and constantan leads were continued into a constant temperature bath, where the constantan was soldered to copper, and the manganin to constantan which in turn was also soldered to a copper wire lead.[1] Of the four soldered junctions three were therefore immersed in the constant temperature bath. All the wire used in the thermocouples was previously tested for uniformity of composition (White, 1914c). The junctions within the bath were held in close contact with the bulb of a delicate thermometer within a test tube which was itself contained within a half-liter thermos flask, and this in turn was inserted into a one gallon cork-insulated porcelain jar (Thermalware); all three containers were filled with liquid paraffin oil. The outermost jar was heated by an electrical heating unit (Hotpoint) with alternating current and controlled by a mercury glass thermoregulator. Each vessel was sealed individually with DeKhotinsky cement. The variations in the temperature of the innermost vessel (normally maintained at about 30°C.) have not been more than a few hundredths of a degree within the duration of any experiment.

The thermocouples were connected to the galvanometer as in the experiments described in the previous paper except that all the wiring was suspended from an overhead copper wire shielding system (White, 1914b). The subject's chair and the experimental table were placed on a zinc plate shield connected to the system; the galvanometer brackets, constant temperature bath, etc., were similarly in electrical connection with the shielding system. In order to exclude automatically all parasitic currents, except those generated close to the thermocouple, an eliminating switch with a resistance equivalent to that of the thermocouple (as devised by White, 1914a) was inserted, and the galvanometer zero was determined with this system in circuit immediately prior and subsequent to any series of observations. The effects of other parasitic currents were evaluated during the process of empirical standardization, which was performed daily; they were quite small, and were allowed for by the use of a correction figure. Temperatures were estimated from the galvanometer deflection as in the previous paper, but records were obtained photographically. Resistances were inserted so as to give a sensitivity of 1° to 2° per cm.

A Hindle camera adjusted to move at a rate of approximately 3 mm. a second was employed. Vertical slits were illuminated by tungsarc lamps and their images focussed on to the camera after reflection from the mirror

[1] In later experiments the manganin has been soldered directly to the copper wire without the interpolation of a short length of constantan. In the initial experiments, the system was constructed symmetrically.

of the galvanometer, which was at a distance of one meter from the camera. The same lights illuminated mirrors on a Jacquet clock, and on tambours connected to automatic and hand signals, transmission being made by air. Air transmission has the advantage of excluding additional electrical circuits.

The thermocouple was in every case mounted vertically above the surface examined, so that the gradients measured are those existing in the direction of the convectional currents. The movement of the thermocouple was controlled by the adjustment screws of a microscope stand; by manipulation of the coarse adjustment contact of the couple with the skin was made. A magnifying glass was used to estimate this as accurately as possible. The thermocouple was then moved by single revolutions of the fine adjustment screw, the pitch of which gave 0.5 mm. vertical movement per revolution, and an interval of about 10 seconds intervened between successive turns. The Y of the thermocouple loop was attached by beeswax to a light fibre v-shaped support; the thermocouple junction was situated between the legs of the V. This support was clamped to the lower end of the microscope tube. A rod extended from the clamp, and compressed a small rubber pipette bulb to a varying extent, so recording automatically changes in the position of the thermocouple.

Though the thermocouples probably had a neglible temperature lag, the same was not true of the fibre support. In general the temperatures recorded during the return of the thermocouple were similar to those observed during its removal from the skin, except for chance errors due to irregular air movements, but some evidence of temperature lag in the system was obtained, since at the intermediate distances the temperatures determined during the removal were usually slightly higher than those observed during the return. The effect of temperature lag was therefore excluded by using a similar rate of movement in both directions, and determining mean values.

We are much indebted to Dr. W. P. White for personal advice and instruction in the use of means devised by him for excluding possible parasitic currents.

Results. The general character of the temperature gradients has been the same in all experiments, but the slope has varied with the dry bulb temperature of the room and the amount of air movement in accordance with general physical principles. Actual values observed over an unshaved area in subject M during a single raising and lowering of the thermocouple in a warm room are given in table 1, the arrows indicating the direction of movement.

The table shows a slight descrepancy between the two series of observations, which is apparently due to a delay in the assumption of thermal equilibrium by the thermocouple and its holder, but both series indicate a

temperature only 0.8° below that of the surface at a distance of 6 mm. The value at this extreme point is not subject to an error from lag, since a full half minute or longer was allowed before beginning to lower the thermocouple and the records demonstrated that the temperature of the thermocouple was no longer altering at the time the return series was started. This series of observations was obtained in a room with closed windows and relatively still air, when the dry bulb temperatures varied between 28.2° and 29.0°, the wet bulb between 22.7° and 23.0°, the dry kata thermometer between 2.4 and 2.1 millicalories per sq. cm. per second and the wet kata thermometer between 11.3 and 11.0 m.c. The effective temperatures on the basic scale (see references in the previous paper) calculated for the conditions at the beginning and end of the experiment were respectively 24.6° and 25.0.° Several series of observations of this type were made on

TABLE 1

HEIGHT	THERMOCOUPLE TEMPERATURE	THERMOCOUPLE TEMPERATURE	MEAN THERMOCOUPLE TEMPERATURE
mm.			
6.0	32.60°	32.60°	32.60°
5.5	32.68	32.60	32.64
5.0	32.79	32.60	32.68
4.5	32.81	32.56	32.69
4.0	32.87	32.58	32.73
3.5	32.93	32.60	32.77
3.0	32.98	32.61	32.80
2.5	33.08	32.70	32.89
2.0	33.16	32.74	32.95
1.5	33.19	32.82	33.00
1.0	33.33	32.98	33.21
0.5	33.39	33.20	33.30
Surface	33.41	33.47	33.44

the same subject and area on this day and the mean values of all the series are given in the uppermost curve for this subject in figure 1 (series designated with *A*). It will be noted that the mean values so obtained differ little from individual values of table 1.

In figure 1 curves are drawn representing the mean values obtained on both subjects on shaved and unshaved areas under varying room conditions including variations in air movement.

In subject M the shaved area was some 3 cm. in diameter and about the junction of the upper and middle thirds of the forearm; the unshaved area investigated was immediately proximal to this; in subject B the shaved area was slightly more proximal and rather larger (4 to 6 cm. in diameter); the unshaved area investigated in this subject was radial to it, except on the

coldest day represented in the figure, where a point distal to it was used (series 4 ●).

Subject B has a moderately hairy forearm, subject M on the other hand has a distinctly lighter hairy coat. In subject B a shorter and finer hair can be observed arising close to the point of emergence of the long and principal hairs; occasionally there are two of such small hairs; in subject M on the other hand such auxiliary hairs can only occasionally be observed, and then only singly. Consequently in an area of 1 sq. cm. of the forearm in the region used for these experiments there could be counted in subject B 25 hairs, and in subject M only 10. The longer hairs were in each subject often some 15 mm. in length. In subject B they formed a well-woven mat some 3 to 5 mm. thick with isolated hairs protruding even further. In subject M there was no such obvious mat; the protection afforded for the first two millimeters was quite light, and beyond this distance the hairs were isolated.

The four upper curves (series 1) represent the mean temperatures observed for each subject on both shaved and unshaved areas on the first experimental day (the warmest) under the conditions already detailed in relation to the data of table 1. Differences between the shaved and unshaved areas may be distinguished in subject B but not in subject M.

The following conditions obtained on the second experimental day (series 2); dry bulb 21.3° to 21.8°, wet bulb 13.3° to 13.6°, dry kata thermometer 4.2 to 4.3, wet 17.3 to 17.4, calculated effective temperatures (basic scale) 17.1° to 17.6°. These values represent the usual environmental conditions in this laboratory. On the third experimental day the conditions for the series 3A were dry bulb thermometer 18.1° to 19.3°, wet bulb 13.1° to 15.0°, dry kata thermometer 6.3 and 6.3 and wet 18.3 to 18.6, and calculated effective temperature 15.1° to 16.2°. During this experiment the windows were open and there were irregular draughts which account for the greater variations in the data. The mean velocity of such movements calculated from dry kata thermometer readings was 0.12 to 0.14 meter per second. On both these days the individual differences and the contrasts between shaved and unshaved areas are in general maintained, though exceptions may be noted.

The two curves of 3B were obtained on the third experimental day after the windows had been closed resulting in a rise of temperature (dry bulb 23.9°, wet bulb 16.1°), and a steady current of air had been produced by an

Fig. 1. Mean of temperatures at surfaces and at various distances from surface, plotted as ordinates against the distance from the skin as abscissae. Environmental conditions for each experimental day detailed in text. Data for subject B indicated by circles (○), for subject M by triangles (△). For data from unshaved areas open symbols are used, from shaved areas solid. Heavy lines have been used to join those data obtained in a strong current of air.

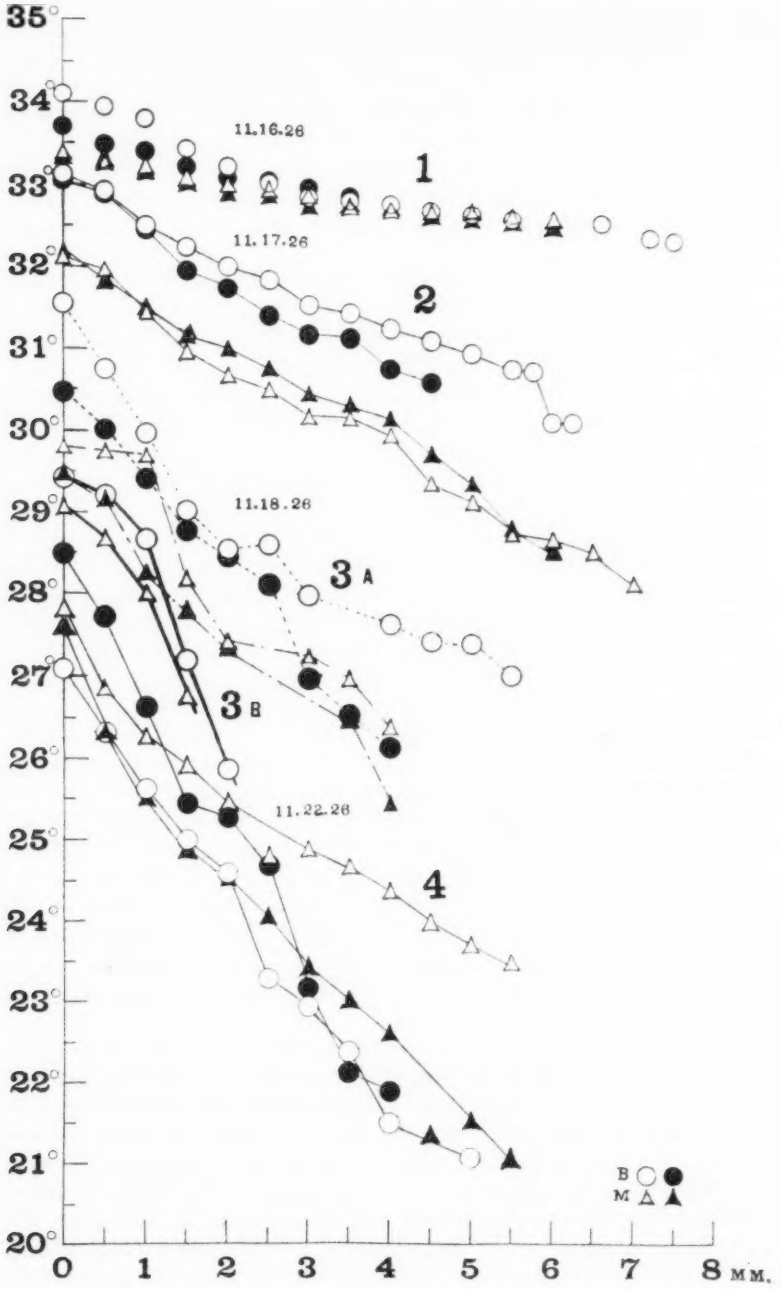
35°
34°
33°
32°
31°
30°
29°
28°
27°
26°
25°
24°
23°
22°
21°
20°
11.16.26
1
11.17.26
2
11.18.26
3 A
3 B
11.22.26
4
B
M
0
1
2
3
4
5
6
7
8 MM.

Fig. 1

electric fan giving a velocity of air across the forearm of about 1.5 meters per second (basic effective temperature 15.3°). Only unshaved areas are represented; the irregularities of the previous curves disappear, but the gradients are increased, and the protection afforded by the hair is obvious in the records. It is indeed remarkable how effective this protection is in both subjects for the first half-millimeter considering the extensive air movement.

The fourth experimental day (series 4) with a lower room temperature and a greater air movement from open windows gave curves similar to those of the third experimental day, but at a lower level. On this day the conditions were as follows, dry bulb thermometer 12.8° to 15.4°, wet bulb 7.6° and 7.6°, dry katathermometer 8.3 to 6.9 and wet 22.9 to 20.7, calculated effective temperatures 9.7° to 11.7° and the estimated air movement 0.18 meters per second. The surface temperatures were in general about 2 degrees lower than those recorded in 3A, and the fall of the temperature in the first millimeter was, in both subjects, 1.5° over the unshaved areas and 2.0° over the shaved areas. The surface temperature of the unshaved area was the lower in subject B, since on this occasion the location examined was slightly more distal, and the variations in surface temperature along the forearm must have been exaggerated (Benedict, 1925a) owing to the great cooling capacity of the air conditions. On completion of these records the fan was turned on as on the previous occasion, but without shutting the windows; the velocity of the air was calculated to be 1 meter per second; the dry katathermometer was 12.3 m.c. per sq. cm. per second and the effective temperature 8.2°. The air temperatures observed are not plotted, as they were similar in type to those shown for experiment 3B. For both subjects the fall of temperature at successive distances from the surface was very rapid. The observed differences between the surface temperature and that of the air at a distance of 1.5 mm. was for the unshaved areas 3.9° and 5.0° for subjects B and M respectively; over the shaved areas the temperature fell 1.22° in 0.5 mm. in subject B and 1.28° in the same distance in subject M. Under these conditions there was such a fall in the general temperature level, that at greater distances the galvanometer swung beyond the scale.

General discussion. The general dependence of the fall of temperature in the surrounding air on the room conditions and on the amount of hair protection is established by the examples given, and the high temperature which commonly exists near the skin is evident even in the shaved areas. Presumably this results from hindrance to air movement through the roughness of the epidermis or the hair stumps. The surface temperature of the shaved area of subject B rarely approaches the low temperature levels observed under similar room conditions in M; since the shaved area receives blood which may have been protected during part of its course by

skin covered with hair, this difference would appear to be that which might be anticipated from the heavier coating of hair.

If the temperature at any point be compared with that of the room, and the logarithm of the difference be plotted against the distance of the point from the surface, the data obtained over shaved areas lie along straight lines (fig. 2), so that they can be approximately represented by the equation

$$\theta_x - \theta_R = (\theta_S - \theta_R)\, \epsilon^{-KX}$$

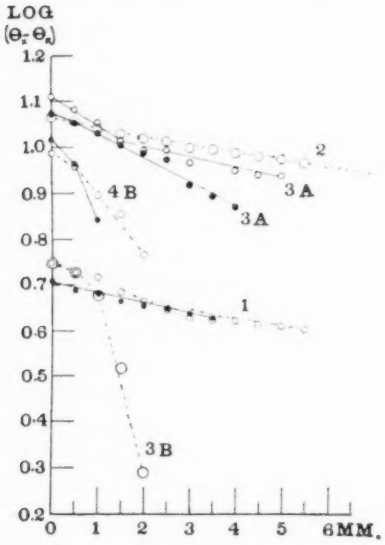

Fig. 2a

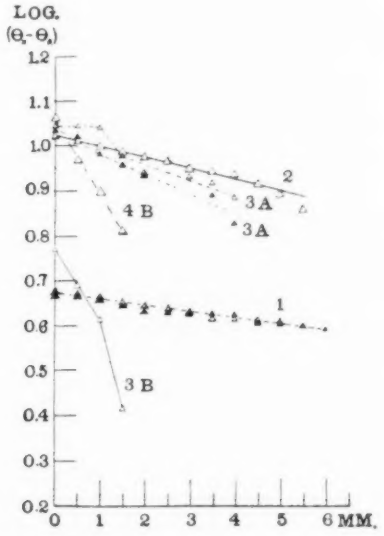

Fig. 2b

Fig. 2. a. Data from experiments on subject B plotted semilogarithmically; ordinates: common logarithm of the difference in temperature of any point from that of the room; abscissae: distance of point from the surface. b. Data from experiments on subject M similarly plotted.

where θ_x is the temperature at any distance x from the surface, θ_R and θ_S represent the room and surface temperatures respectively, and K is a constant varying with the conditions of the experiment.

The temperature gradients that exist above the unshaved areas may be similarly represented, except that there is often a definite change of slope at a distance of 1 to 2 mm. (occasionally 3 mm.) from the surface at a position about that of the margin of the hairs, if a few isolated protruding hairs be excluded. This definite change of slope is also visible, though perhaps less clearly, when the data are plotted arithmetically.

In warm rooms the change in slope was usually slight, and the regularity of the curves was indicated by a single temperature measurement made on subject B (series 2) at a distance of 40 mm. By extrapolation of the curve drawn through observations made up to a distance of 6.5 mm., the temperature at 40 mm. was estimated to be 23.6° or 9.5° below the surface and 2.0° above the room temperature, the actual observed temperature was 23.5°.

In a cold room (i.e., cold in relation to the habitual environment of the individual) with a cross current of air produced by a fan, as in experiment 3B, the thermal gradient was usually less steep within the area protected by the hairs and the contrast can be demonstrated by both methods of plotting. In subject M with sparser hair the contrast was less marked and sometimes absent. Even over the shaved areas in both subjects the gradient may under these conditions be less steep within the first half millimeter, presumably owing to some hindrance to air movement due to the contour of the skin, to irregularities on its surface, or to stumps of hairs inadequately removed.

In a cold room without artificially increasing the air movement, the gradient within the protected area was sometimes steeper than that observable at a greater distance, and, if plotted semilogarithmically might give a greater value for K. With somewhat warmer conditions the break in the curves usually disappeared. Sometimes however changes similar to those observable in the fan experiments could be distinguished; in artificially warmed rooms the air movement was occasionally increased, probably as the result of the interaction of warmed and cooled air currents.

The surface temperatures of the shaved areas were, as might be expected, somewhat lower than those of the unshaved, and the air adjacent to these surfaces also had a lower temperature. When, however, the data were plotted semilogarithmically, the slopes of the curves and values of K were greater over the shaved than over the unshaved areas except in warm conditions, when they were almost identical.

The variations due to the hairs can probably be explained on the basis of air movement; vertical air currents due to thermal changes must be hindered by the presence of hair, and these currents, if present, would tend to minimise the thermal gradients. On the other hand the hairs must to some extent protect the area from cross currents of air and such currents would tend to exaggerate the thermal gradients. The two types of air movement, having opposite effects on gradients, would both tend to increase heat loss, so that no simple relationship of heat loss to gradient is to be anticipated.

SUMMARY

1. A method is described for the determination of the temperature of the air adjacent to the surface of the skin in man up to distances of 5 to 6 mm.

2. It has been demonstrated that under conditions classed as comfortable or warm the layer of air 1 mm. distant from the surface is less than 1°C. below that of the surface.

3. Under colder conditions the layer of air 0.5 mm. from the surface is less than 1°C. below that of the surface except occasionally, when the surface is shaved and is subjected to tangential air movement up to 60 meters a minute.

4. In consideration of this layer of warm air, and the low thermal conductivity and capacity of air, it is unlikely that exposure to air introduces any serious error into surface temperature measurements made with thermocouples which protrude only slightly from the surface, such as those described in the paper immediately preceding this.

5. The effect of the protecting layer of hairs on the thermal gradients in the air, and the differences existing between shaved and unshaved areas is discussed. The hairs allow even exposed parts of the body to have a special environment and almost to maintain a "private climate," similar to that described for clothed areas.

Acknowledgments for helpful suggestions given us by Dr. F. G. Benedict and by Dr. W. P. White have already been made in the previous paper. It is a pleasure to acknowledge that our interest in the air to surface thermal gradient was aroused by a conversation with Doctor Benedict.

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THE FORM OF THE RECORD OF THE ACTION POTENTIAL OF VERTEBRATE NERVE AT THE STIMULATED REGION

GEO. H. BISHOP

From the Department of Physiology, Washington University School of Medicine, Saint Louis

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This paper comprises an investigation of the form of the potential wave accompanying the excitation of nerve freed from all the distortions we have been able to detect. Previous papers from this laboratory have given an analysis of the compound wave as recorded from the nerve trunk and from its roots, and have described the changes the potential wave undergoes with propagation (Erlanger, Bishop and Gasser, 1926, and previous papers). There have also been reported a considerable number of factors in the nerve itself and in the recording apparatus that tend to distort the potential record from the nerve (Bishop, Erlanger and Gasser, 1926a). The first section of this paper describes a method and apparatus for recording the potential of nerve by use of a Wheatstone bridge, in which certain of these distorting factors in the nerve can be balanced out. The second section presents certain data upon the form of the electrical phenomena of nerve, and upon the response at the site of stimulation.

So far as we know this subject demands no comprehensive survey of the literature, further than to point out that previous methods of recording have not been suitable for this investigation. An induction shock of adequate strength to stimulate applied at a nerve lead, might break the string of the average string galvanometer, and would distort the capillary electrometer record beyond recognition. The differential rheotome might be employed, but this rather tedious technique has practically been abandoned. Even the inertialess cathode ray oscillograph registers distortions arising in the nerve itself, that have to be eliminated to obtain significant records.

A. Method of recording at site of origin of potential. Since the nerve trunk consists of fibers having different propagation rates, the record of the action potential wave led off at any point except at the locus of stimulation is a record of summed axon action potentials which are individually in different phases. The true time-potential curve of the nerve fibers' activity is therefore masked. Using the cathode ray oscillograph, the action potential at the point of origin can be recorded by em-

ploying the stimulating cathode as one of the nerve leads to the recording apparatus. It has been found that all fibers in the same nerve, though having different rates of propagation, still exhibit the same duration of potential response at a given point on the nerve (Erlanger, Bishop and Gasser, loc. cit.). Under the stimulating cathode, therefore, if the stimulus is a very brief one, all the fibers may be inferred to be in approximately

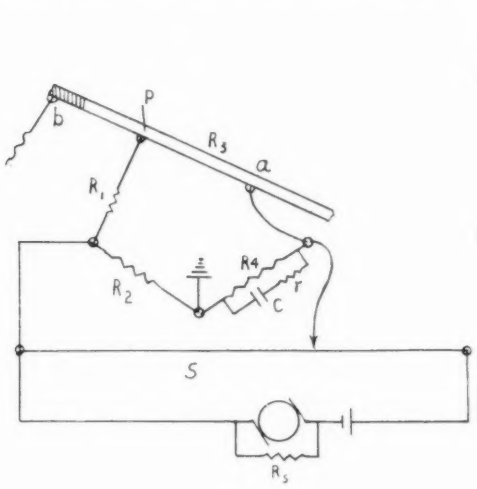

Fig. 1

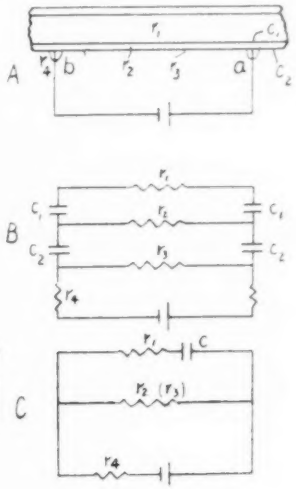

Fig. 2

Fig. 1. Wheatstone bridge diagram for compensation of electrical stimulating potentials in nerve. *a*, anode, *b*, dead end lead to grid of amplifier, *p*, cathode and live side lead through R_1R_2 to ground. S, potentiometer current supply. For discussion of use see text.

Fig. 2. Analysis of physical circuit through nerve in terms of capacity and resistance. A, diagram of nerve and stimulating circuit. B, diagram of above as resistance and capacity. C, simplified diagram whose reactance can be made approximately that of B. *a*, anode, *b*, cathode, c_1, effective capacity of axons, c_2, effective capacity of connective tissue, r_1, ohmic resistance of axons in series with axon capacity, r_2, resistance of connective tissue and leak through axon capacity, acting as a shunt to c_1, r_3, resistance of salt solution on nerve surface, shunting c_1 and c_2, r_4, resistance of electrodes in series with the whole.

the same state of activity at the same time. The technical difficulty of recording such an action potential lies in the distortion of the record by the stimulating current, which distortion lasts over into the excitation phase (Bishop, Erlanger and Gasser, loc. cit.).

This "escape" distortion appears to be due in large part to the electrotonic currents initiated by the stimulus, applied to a polarizable core conductor such as nerve. That is, it is the record of the polarization, by the

electrical stimulus, of some constituent of the nerve trunk. In a metallic model of the same resistance distribution between leads the phenomenon is not exhibited. It can be virtually eliminated in nerve by placing the

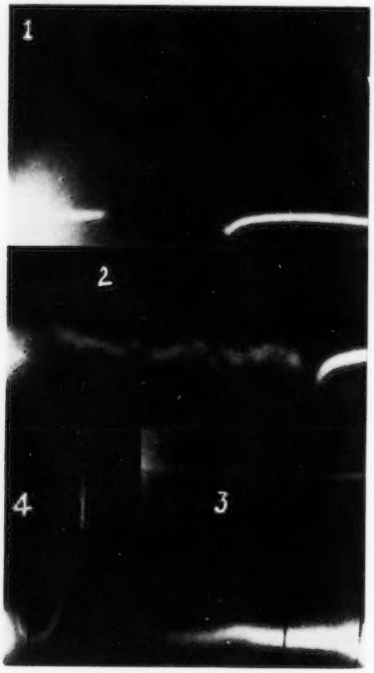

Fig. 3. Braun tube records from nerve. *1*, record of galvanic current applied to nerve, lead from cathode, only slightly compensated. The deflection represents 7 millivolts at the cathode, followed by the counter E.M.F. of polarisation. *2*, galvanic current compensated in bridge by non-reactive resistance in arm R_4 of figure 1. Just below threshold, much stronger current than above, as can be seen from the record of the polarisation at start and end of current. Ripples in line due to imperfect contact on commutator wheel during make. *3*, bridge balanced by non-reactive resistance in place of the nerve, same current as above, break only shown, as test for effects of capacity in apparatus other than that of nerve. *4*, action potential, galvanic stimulus, compensated in bridge by both resistance and capacity, lead 4 mm. from cathode, low repetitive second wave. The effect of capacity balance in arm R_4, figure 1, is to reduce both the initial and final polarisation distortion and also the irregularities due to imperfect contact during current flow.

nerve in one arm of a Wheatstone bridge, and so balancing the bridge (fig. 1) that the stimulating cathode proximal to the amplifier-cathode ray oscillograph leads is at the same potential as the opposite point of the

bridge. This opposite point is grounded, and serves as one lead to the recording apparatus, the other being on the killed end of the nerve.

It is obvious that the complicated structure of the nerve, which, as will appear below, forms part of the arm of the bridge R_3, and also part of the arm R_1, makes the bridge more difficult to balance than if, for instance, we were balancing a single polarizable resistance such as a simple membrane in one arm. As is well recognized, a semipermeable membrane polarized by a current can be treated as a condenser; it is in fact a form of electrolytic condenser in which the concentration of ions at the surfaces of the membrane corresponds to the pressure of electrons in the plates of an electrostatic condenser. The material of the membrane itself is in this case the dielectric, its surfaces are the plates. The resistance of the solutions corresponds to a resistance in series with an electrostatic condenser, and the leaking through of ions to which the membrane is permeable, if any are in the solution, corresponds to a shunt across the plates of the electrostatic condenser. In a tissue a further leak takes place around the polarizable cell surfaces, through the fluid-filled interstices between the cells. Such a condition can be diagrammed tentatively as in figure 2.

In a Wheatstone bridge, therefore (fig. 1), such a tissue requires for a balance, first, a capacity in the adjacent arm corresponding to the electrolytic capacity of the membranes; second, an ohmic non-inductive resistance in series with this capacity, corresponding to the ohmic resistance of the electrolytes of the system, in series with the membranes; and, third, a similar resistance shunting these, corresponding to the leak through or around the membrane if any takes place. Records of these conditions appear in figure 3, of the nerve balanced against a non-reacting resistance (record 2), leaving a record of the nerve's polarization, the same bridge with a non-reacting resistance of equal value in place of the nerve (record 3), and with the nerve's polarizability balanced out by a reacting condenser-resistance unit in the opposite arm, leaving only the action potential wave (record 4).

If two similar membranes are in series with respect to the lines of current flow, the effective capacity of both will be one-half of that of each separately, etc., quite as in the usual type of condenser. If the two or more effective capacities are in series but unequal in value, the value follows from the equation $\frac{1}{C} = \frac{1}{c'} + \frac{1}{c''} + \frac{1}{c'''}$, etc. If the two capacities are in parallel, however (fig. 2, C_1 and C_2, charged through $r_1 + r_2$), they cannot be balanced in the bridge by a single capacity, for the charging of these two capacities through their respective resistances will in general take place at different rates; the resultant curve of potential rise having the properties of the sum of two logarithmic curves with different time constants. Any single condenser balanced against this combination will fill according to a

simple logarithmic curve, and will therefore compensate in the bridge only one of the unknowns, leaving as a residuum a wave of potential which is characteristic of the other. Experience shows that this is the situation in the nerve trunk, the polarizaton of which can be balanced by two variable condensers better than by one. Generally, however, a one-condenser balance leaves a residuum which is not a serious distortion of the record, when so adjusted as to split the differences between the two or more components of the nerve's polarization.

An examination of the nerve as arranged on its electrodes should make the situation clearer (fig. 4). When a stimulating current is passed across the bridge, the path through the nerve is from the electrode at *a* partly along the wet surface of the connective tissue sheath, partly through this sheath longitudinally, and partly through the sheath transversely and into the nerve proper. The sheath is polarized characteristically by the latter component. This transverse current then again divides, part passing down the nerve between the axons and part polarizing the axons, with a current down the axon core. It may be noted here that it makes relatively little difference whether the axon surface is semipermeable or completely impermeable to ions, that is, whether the "condenser" is "leaky" or not, it will acquire a counter E.M.F. in either case, and in either case at least a transient current will flow along the inside of the axon. The longitudinal currents may or may not meet with further polarization (e.g., at the connective tissue cells, or at nodes of Ranvier) before they emerge from the nerve at the electrode *b*, which is also one lead to the recording instrument. At any rate the current redistributes itself in the nerve at the region of *b*, the surface component flowing off directly, the inner components polarizing the cell membranes in the reverse order to that at *a*. A component of these "core" currents will tend to flow along or around the polarizable resistance across the nerve, toward *c*, the other lead. (This component is of course present at *a* in the reverse direction, but may be ignored for present purposes.) The path of this current will be along the nerve, through its membranes, and back along the wet surface to the electrode *b*.

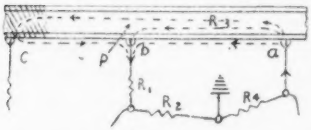

Fig. 4. Analysis of balance in Wheatstone bridge for a point inside the nerve, which in effect puts a part of the nerve's polarisation into each of two compensating arms, and leaves this point at a constant potential with respect to the killed end lead when current is applied. *a*, anode; *b*, cathode and live lead for the action potential; *c*, dead end lead, maintained in balance for applied potentials with *p* inside the nerve and with the ground lead opposite. Polarisation at *a* and *b* tends to balance each other in the arms R_1 and R_2. If the lead *c* is connected instead to *b* the polarisation is all in the arm R_3, and may be compensated in R_4. The indifferent lead is then at *a*, which may be killed.

A branch of this pathway leads past c, and thus the lead on the killed end of the nerve is on a potential gradient from the anode a to the cathode b, when a current is passed through the nerve. Under the electrode at b, however, there is some point P in the potential gradient from inside to outside that at a given time will have the same potential as the lead c. If the two paths through these points P and c were similar, and if we could make our other or grounded lead to the recording mechanism from this inaccessible depth P, in the nerve, the stimulating potential would affect both leads alike, and would, therefore, not be recorded. The resultant action potential, however, would be recorded in the usual sense, as a difference of potential between b (or P) and c.

We may virtually ground this point P inside the nerve by proper balance of the bridge, such that its potential is that of the ground opposite. This is done by trial and error in the arm R_4, while observing the visible record of imbalance on the Braun tube after amplification. When balanced, the action potential upon excitation may be looked upon as a potential imbalance of the bridge, or as a potential lead in the usual sense, from the killed end to the amplifier grid, and from the live side through the bridge to the ground. There remain to be considered, first, the possible effects of this reacting bridge circuit in distorting the potential led off through it; second, the effect of the physical change in the nerve with stimulation upon the bridge balance, and third, the effects of the battery or induction coil circuit reactance upon the bridge circuit.

1. *Distortion of action potential by bridge reactance.* As the nerve becomes negative upon stimulation at b, the distributed capacity of the length of nerve between c and b and of the amplifier grid lead at c discharges across the bridge to the ground. The reactance of the bridge will distort the record of this potential change in proportion as the current involved is large compared to the reactance of the bridge. The nerve, however, has a high resistance, and the grid capacity is extremely low, while the non-reacting arms of the bridge can be made low. They then form a shunt for this current from the nerve, around the high resistance reacting arms of the bridge. The effect of the bridge on the form of the potential wave can thus be made insignificant.

2. *Effects of change in physical state of the nerve with activity.* It is generally presumed that cells alter in electrical permeability upon excitation. In nerve, this hypothesis has been elevated to the status of a theory of stimulation. If such alteration takes place during stimulation, the balance of the bridge described above will be impaired by reduction of the nerve's polarizability. This will scarcely be appreciable when induction shocks are used, since the applied current ceases almost before the response commences, after which the bridge acts merely as a lead from the nerve surface. With galvanic currents which outlast the excitation, a potential

from the galvanic source will sum with the action potential if the bridge is transiently imbalanced during the excitation. This to be sure will only be an accentuation of what happens under a simple nerve lead; if the permeability changes, whatever electrical reaction may be going on at the nerve surface or inside will alter in apparent intensity with alteration of the effective resistance. Records made just below threshold give no sign that *during the process of stimulation*, before threshold of discharge has been reached, any such reduction in resistance takes place. In fact the *apparent* resistance increases, by reason of the counter E.M.F. of polarization, but this change follows closely the decremental formula for a simple polarization structure. If any such change takes place *during the response*, the use of this bridge for galvanic current stimulation should cause some fraction of the potential applied at the cathode to sum algebraically with the action potential. If now such a galvanic current is shortened in duration until it ceases to flow just after stimulation, and just as the response starts, this summation of action potential and applied potential is done away with. Experiments so performed show no material change in form or size of the wave as the duration of stimulus is so shortened. Cathodal polarization, to be sure, reduces the amplitude of potential in general, but this reduction takes place only *after* the *first response*, to currents of ordinary stimulating value, and is presumably due to interference with the electrochemical reaction in the nerve involved in the response, or in recovery (Bishop and Erlanger, 1926).

3. *Effect of coil reactance on bridge.* When a galvanic current is employed to stimulate the nerve through this bridge, the relatively long leads to the circuit breaker have considerable capacity. This capacity of each lead discharges to the ground and to the grid *b* (fig. 1) at make and break of the circuit through its respective arms of the bridge, and thus affects the record, since the two battery leads will in general have different capacities and potentials. This effect can be compensated by adjusting the capacity of the lead wires, but this is usually not necessary since the discharge is so short. A resistance relatively low compared to the bridge, but high compared to the slide wire (R_s fig. 1), shunting the commutator reduces this capacity effect. By analogy, however, we may infer a similar effect of the capacity of the induction coil used to stimulate, especially since as usually wound, the coil is asymmetrical. The capacity of the wire in the coil will be distributed linearly, while the induced potential will be highest near one end of the wire, that wound on the inside of the coil, since this is nearest the primary coil. The coil will, therefore, tend to discharge into its own capacity, which will act like a shunt to ground from some point of the circuit.

Some such induction effect as this in stimulation by shocks is more troublesome than capacity effects in galvanic current leads, probably be-

cause of the higher potentials developed in the coil. Its distorting effect may be very greatly reduced by connecting two secondary coils in series, but so connected that the end of the wire from the innermost turns of one coil is joined to that from the innermost turns of the other coil. The current then passes through one coil from the peripheral to the central and through the other from the central to the peripheral winding. The primaries are then joined in series in such an order that the induced potentials in the secondaries act in the same direction in the stimulating circuit. If the two coils are alike and stand in the same inductive relationships to their respective primary coils, the capacity-inductance arrangement is symmetrical. Once set in this relationship, the strength of shock is regulated by the current flow through the primary coils. It should be noted

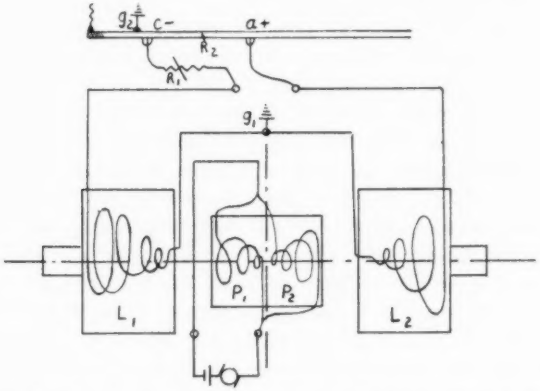

Fig. 5. Diagram of symmetrically wound coil which may be used as a Wheatstone bridge to obviate large shock distortions from records taken close to the stimulating cathode. See text.

that this arrangement does not abolish the escape, which is due to relationships between the reactivity of the coil and the reactivity of the nerve as a polarizable conductor, but it enables it to be controlled or compensated more intelligently.

4. *Record through an induction coil bridge.* A coil has recently been constructed having approximately the constants of the Porter coil (fig. 5) but wound symmetrically. The primary is centrally located and consists of two halves the mirror image of each other, but with current flowing in the same direction through each, either in parallel or in series. It turns about a transverse horizontal axis. The secondary consists of two parts placed on either side of the primary; these slide along their longitudinal axes. The windings are in opposite directions to be symmetrical about the primary, and they are so connected that their induced potentials sum

in the same direction. Turning the primary regulates the induced voltage, and reverses the secondary potential, while by sliding the secondaries their inductive and capacitative coupling with the primary can be altered.

Such a coil in itself may be used conveniently in leading from or near the stimulating cathode, as a roughly balanced bridge. This bridge consists of two arms, L_1 and L_2 (fig. 5), with equal resistance and inductance, grounded at their junction g_1; and two other arms R_1 and R_2 (the latter resistance of the nerve) which can be equalized by adjusting a variable resistance in the cathodal lead, R_1. If the nerve is of uniform structure, the polarization at the anode and cathode tend to balance each other, being in opposite arms of the bridge, and some point inside the nerve is held at the potential of the ground g_1. This involves leading off the action potential through the inductances L, which will distort it in proportion as the change of potential is rapid. A second ground lead below the cathode at g_2, which can be as close as desired to the cathode, makes the effective lead at this point, but interferes with a perfect balance by throwing the polarization at both anode and cathode into one arm of the bridge. However, in a Wheatstone bridge capacity (i.e., polarization) can be balanced against inductance, which can be accomplished in this case by inserting a small iron core part way into the appropriate secondary coil. A ground lead can then be taken from the cathode itself with but slight distortion of the record by the shock.

5. *Compensation circuits to recording leads.* Instead of balancing such a coil as a bridge the same effect can be obtained by leading through high resistance shunts from various points of the coil circuit to the amplifier grid. Since one lead of our input is to the ground, this can be ignored, except in so far as its resistance will occasion a potential drop between the ground and the nerve whenever a current flows through it. Such potentials will appear as components of the so-called escape distortion of the shock record. Each factor in this escape will have its origin in the circuit through the stimulator and nerve, and the problem is to pick out such factors and apply them to the grid in a direction which opposes the distortion as recorded, thus compensating the leading off circuit as far as the stimulating current is concerned.

The disturbance recorded from the shock potential consists in general of three factors; that due to the break of the primary current, recorded through air or through capacitative coupling of primary and secondary; that due to the secondary shock itself, discharging over the ground electrode resistance; and that due to the polarization of the nerve by the secondary current. (See also Bishop, Erlanger and Gasser, 1926a.) The first can be compensated by leading a wire from the appropriate primary coil terminal to end in the air in more or less close proximity to the grid input (fig. 6, *A*). If connected to this input through a resistance of up to

10^9 ohms its effect is too potent. The second, or shock effect, is compensated by leading from one secondary terminal to the grid through about 5×10^5 ohms, a resistance which will, of course, vary with conditions. The third or polarization factor may be compensated by leading from the secondary terminal to the grid through a resistance of the same order as in the preceding case, in series with a capacity corresponding to the effective capacity of the nerve circuit, which may be anything up to 0.1 Mf. if the nerve has a sheath. This series resistance is so high that the reactance of the condenser does not appreciably affect the nerve action potential record when shunted by the nerve itself. These circuits are diagrammed in figure 6 *A*, together with an analysis of the components of the escape distortion (6 *B*). The circuits to the grid electrode will be from anode or cathode of the secondary, depending on the relation of the coil reactance to the nerve reactance.

The adjustment of these circuits to any degree of precision is a tedious

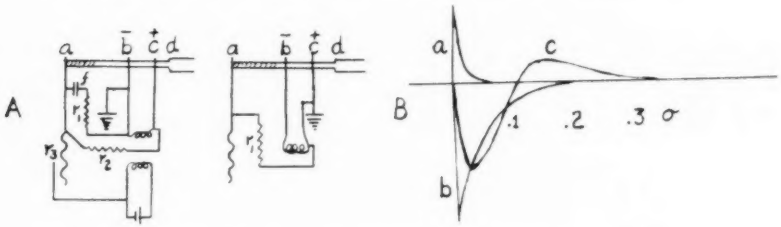

Fig. 6. A, diagrams of leads to compensate shock distortions by applying equivalent potentials to the grid lead in a direction opposite to the distortion recorded. B, essential form of elements of the shock and polarisation potentials as usually recorded from induced current stimuli.

process, and one which would be even more difficult without a visible record coincident with the necessary manipulations. Their approximate balance, however, is rather simple, and for routine practise the reduction of large shock distortions to negligible values is quite feasible. It may be suggested, furthermore, that each complexity of apparatus only corresponds to a complexity in the nerve itself, and thus represents a datum in our knowledge of nerve. Certainly the ratio of these complexities has not yet exceeded unity.

B. Form of the potential wave. It is obvious that with these circuits, the record of the action potential wave may have eliminated from it not only extrinsic distortion, but also the polarization of the nerve axons by the stimulating current (fig. 6 *B*). This polarization may be an essential event in stimulation. At any rate the action wave as thus recorded is the *result* of stimulation and does not include the stimulation process; it is a measure of the nerve's reaction alone. In such a record the potential of

excitation may be seen to rise from zero (that is, from the level of polarization of the nerve by the stimulus, which is balanced out) and, allowing for change of phase relation in different fibers with conduction, the axon potential may be inferred to be of the same general form throughout conduction, as at the site of stimulation (Erlanger, Bishop and Gasser, loc. cit.).

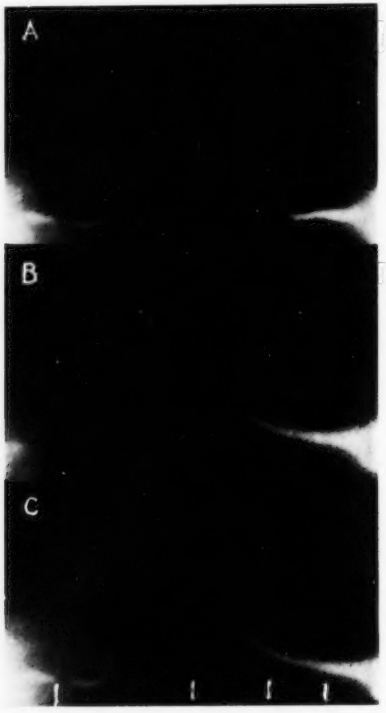

Fig. 7. Records of action potentials from a bullfrog sciatic motor root at 22°C. A, record from cathode of stimulating coil, escape approximately compensated except for short primary effect recording the time of start of the stimulus. The form of the falling phase is distorted in one direction by a second lead through the coil from the adjacent anode, and in the other direction by an effective diphasic lead at the killed end. B, record from anode, cathode 3 mm. distant, in depressed region near the killed end, distortion chiefly in the rising phase. C is a composite of the rising phase of A and the falling phase of B, from which figure 8 is plotted. Time in 0.5 sigma.

In general, the potential rises along an S curve. As we observe nearer the stimulus, however, the shape of the composite wave changes characteristically, approaching that of the axon wave. Aside from the rise becoming steeper (due to phase relationships of the constituent axons),

the initial upward curvature becomes shorter, the point of inflection falling nearer the start. At the stimulating cathode the rising phase finally approximates to a decremental curve of potential increase, that is, the rate of increase of potential is at a maximum very shortly after the initiation of the response, though a very short initial increment cannot be excluded[1] (fig. 7). The axon response then assumes the character of an explosive reaction. Several factors contribute to an apparently longer inductive period and more pronounced S-shape away from the stimulating cathode.

The first of these factors is the falling out of phase of axon processes travelling at different rates. Even if we assume the rise of potential to be rectilinear, that is, picture the potential wave as a triangle, the distribution of fibers according to size, and therefore, rate, which in the components of the alpha wave, for instance, approximates a simple chance distribution curve, soon brings the few fastest processes forward from under the main crest (Gasser and Erlanger, 1927). This will be even more effective if each axon process has a short upward inflection at its start, instead of being rectilinear.

A second factor which contributes to the S-shape of the rising potential anywhere except at the site of stimulation is the spread of potential ahead of the excitation which must theoretically take place in a structure such as nerve. There has been reported (Erlanger, Bishop and Gasser, loc. cit.) an attempt to measure this potential gradient, but the method of arriving at the value leaves a wide margin of error, and the figure (0.07σ) is approximate. If a constant potential is applied to nerve the potential gradient from the electrode along the extra-polar stretch is such that the potential falls to half value for each 2 mm. of distance (Bishop and Erlanger, loc. cit.). This figure suggests an appreciable gradient from a steeply rising action potential. At the site of stimulation this component of the record is eliminated since there is no preëxisting potential wave to cause it, and

[1]Fulton (1926, p. 88) in analyzing an early record republished from Erlanger and Gasser obtained by subtracting a large escape from the combined curve of action potential and escape, takes a curve which the authors extrapolated by a row of dots, to be their interpretation of the shape of the initial rise of potential. Fulton then, reading this curve as if still further extrapolated to the base line, makes the following comment, in which the italics are his. "Note that the ascent of A (the curve in question) is *not* sigmoid." On taxing the authors in question with this interpretation, they state to the present author that, in their estimation, the curve may have been sigmoid, that it probably was sigmoid, that the record was not published to determine this fact, and that the dotted line extended part way to the base line was intended by them to convey their lack of certainty to the reader. Their results are therefore not in conflict with the present findings, in which the escape has been largely suppressed from the record.

In fact the authors state in regard to this figure, that "The very beginning of the action current here is not determinable because of the steepness and faintness of the records in that position" (Erlanger and Gasser, 1924, p. 657).

the polarization from the stimulating current which might be looked upon as its counterpart under the electrode may be balanced out in the bridge circuit.

Finally, the S-shape is further induced by leading from the nerve through the connective tissue sheath, which in effect broadens the electrode contact with the nerve. This sheath may be removed from such a nerve as the dog phrenic, or a bullfrog motor root may be employed. The latter gave the records of figure 7, replotted onto linear coördinates in figure 8.

To infer from the nearly exponential form of this curve of rise anything

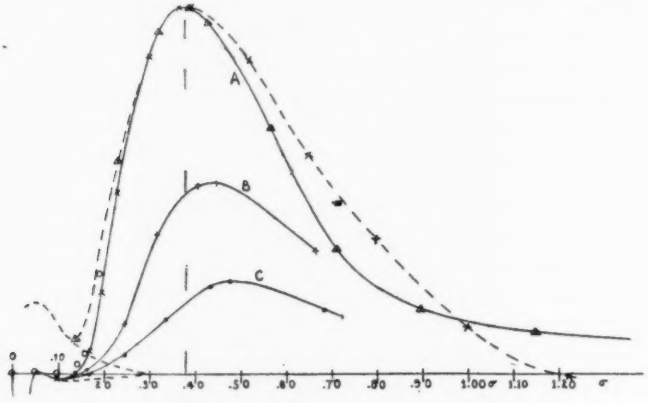

Fig. 8. Curve A, replot on linear time coördinates of record 7 C. The dotted curve is the rising phase of record 7 B and the falling phase of record 7 A. Curves B and C, plots of submaximal records taken in same series as 7 A, with change only in strength of stimulus. The curves start at about the same time, but the crests are later since not all axons are stimulated simultaneously. The "latent period" or effective duration of the shock is longer for the coil here used (0.10σ), than for the Porter coil (0.06σ), the time constant of its discharge curve being about twice that of the Porter coil in a similar circuit. The slight artefact apparently remaining under the start of the wave (dotted line) does not change appreciably with change in strength of shock.

about the monomolecularity of its underlying chemical processes, is hardly justifiable. Any number of curves might approximate thus closely to logarithmicity, and the nerve is probably not so simple that its activity can be encompassed in terms of a single mathematical function. However, one characteristic that stands out in these records is that the *rate* of *change* of potential is highest remarkably soon after its start, after which it decrements progressively. The potential is increasing at its most rapid rate within 0.000,05 second after the first detectable sign of potential response to stimulation. On the other hand, the effect of a threshold stimulating galvanic current, upon irritability, as measured by the strength

of a superimposed induction shock necessary to sum to an earlier threshold, is greatest at the start of the current, and just before the response to the galvanic current the irritability is changing very slowly (Bishop, Erlanger and Gasser, 1926b). We thus have a condition in which the slowest change of the stimulation process very immediately precedes the fastest part of the response process.

This condition may have some bearing on the membrane theory of nerve stimulation. This theory involves the assumption that stimulating agents, including electrical current, change the permeability of the cell membrane, permitting excitation by allowing reaction between diffusible substances previously kept apart. Considering the fact that change of

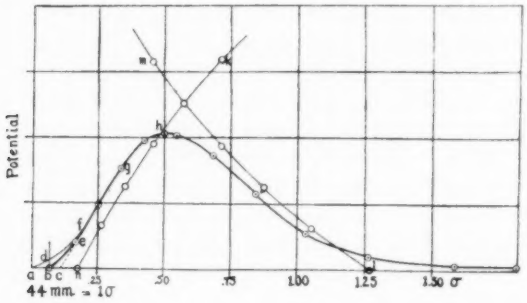

Fig. 9. Schematic analysis of action potential wave. *n-k*, exponential curve or rise parallel to *ceg. m-l*, mirror image of this curve about a horizontal line through a point above the crest *h*, which gives a triangle with *n-k* approximating the potential area. The whole has been displaced later to separate it from the potential record. *b*, start of process as recorded from the stimulating electrode. *a*, start of potential curve due to flow of current ahead of excitation, after propagation. If the distance along the X axis is read in millimeters, the wave is the instantaneous standing wave along the nerve during propagation.

permeability to ions is not in evidence during the excitation phase *preceding* response, an abrupt change in permeability, at the threshold, corresponding to such an abrupt change of potential as the nerve exhibits, would be surprising. One would expect the change in permeability to progress more or less in proportion to the quantity of current flowing. The distinctively explosive character of the nerve's threshold response seems more characteristic of a chemical reaction in a metastable system, the initiation of which, at a critical potential between the system and its surface acting as an electrode, might be quite independent of a change in interfacial permeability.

The falling phase of the potential wave of nerve. A quite arbitrary but convenient diagram for standardizing the action potential may be derived

as follows. The logarithmic curve that most closely approximates the rise of potential (fig. 9, *n k*, approximating a part of *b e g h*) is prolonged to a point above the crest of the potential wave. About a line through this point, parallel to the X axis, the mirror image of this curve is drawn (*m l*). The prolongation of this curve coincides in general with a considerable part of the falling phase of the wave. Whatever significance, if any, this circumstance may have, the area so bounded serves as a convenient approximation for an action potential wave. That this procedure is justifiable for obtaining a picture of the rising phase of the nerve's response might be argued from the data presented in this paper. The same justification does not hold for shortening the falling phase, though certain sources of error also affect its recorded form.

One of the factors that should affect the apparent form of the rise, also holds for the falling phase, namely, the flow of current and consequent spread of potential along the polarizable nerve surface from the excited region (for the rise, diagrammed in *a d f*, fig. 9). Lillie infers that this would assist in the recovery process, as it probably does in the steel wire model (Lillie, 1922). Owing to the relatively flat gradient of the fall of potential as compared to the rise the effect should be less here. Other sources of error, however, of the nature of distortion of the record due to the structure of the nerve, and to the conditions at its killed end between the leads to the recording instrument, have been discussed elsewhere (Bishop, Erlanger and Gasser, 1926).

These sources of error can be largely obviated by stimulating near the killed end, between leading off electrodes, conduction being in the reverse to the usual direction (fig. 7*b*). The potential wave has then subsided at this killed end before it does at the live lead, and whatever distortion there is enters into the rising phase instead of the falling phase. A shock between leads is rather difficult to balance out, and the rising phase is, therefore, further distorted by the shock escape. If a nerve root is employed, absence of a sheath reduces to a minimum the spread of potential from the excitation back toward the stimulating cathode, as the wave subsides under the ground lead.

Such a potential wave from a bullfrog motor root is recorded in figure 7*b*, after conduction 3 mm. from the cathode, and its falling phase is probably as true a picture as can be obtained. Since the other wave of figure 7*a* taken at the stimulus, has a distortion in the falling phase due to conduction via the anode to grounded cathode, as the wave passes the anode, and a further diphasic artefact assignable to the conditions at the killed end of the nerve (Bishop, Erlanger and Gasser, 1926), the falling phase of this record of figure 7*b* has been combined with the rising phase of the record of figure 7*a* in the diagram of figure 8, the whole replotted to linear coordinates. If allowance is made for conduction, the crests fall together,

as well as the second halves of the rising phases, and no other corrections are to be applied that we know of at present. The form is not materially different from the average record taken at the stimulus in the usual way, when reasonable precautions have been taken to avoid artefacts, and may serve for purposes of comparison in detecting the presence of such artefacts. A potential persists long after the main action wave is over with. This long lasting potential may be not a part of the excitation phenomenon proper, but a sign of some recovery or restoration process. The form of the wave suggests that the excitation process returns to a different base line, i.e., to an altered condition of the nerve, from which recovery is very slow compared with the subsidence of the excitation itself.

SUMMARY

Methods are described for eliminating the stimulating potential effect from the record of nerve response to galvanic or induced currents. These methods involve balancing out the distorting potentials by modifications of the Wheatstone bridge principle, taking into account the physical properties of the nerve as a conductor, and the reactance of the stimulating circuit. Relatively undistorted action potentials may thus be recorded from the cathode of the stimulating circuit as a lead.

At the site of the origin, the potential record rises abruptly with a very short induction phase, and decreases in rate of change of potential toward the crest, the rising phase describing a very asymmetrical sigmoid, which approximates to an exponential curve. The falling phase can be described for purposes of analysis as the sum of two exponential curves, with quite different time constants, such that the slower curve in effect constitutes a new base line to which the faster curve falls asymptotically. A negative variation of appreciable magnitude persists for at least ten sigma after excitation. The subsidence of two processes at quite different rates would be consistent with the form of the potential, but is not demonstrated.

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ACTION POTENTIALS ACCOMPANYING THE CONTRACTILE PROCESS IN SKELETAL MUSCLE

GEORGE H. BISHOP AND ARTHUR S. GILSON, JR.

From the Department of Physiology, Washington University School of Medicine, Saint Louis

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The monophasic action potential of skeletal muscle as usually recorded consists of a simple wave somewhat similar to that of nerve, but of longer duration. Deviations from such a simple form, however, in the nature of second maxima, have suggested to various authors that the electromyogram was associated with the chemical process underlying contraction. This interpretation receives support by analogy with the T-wave of the electrocardiogram, and from the fact that the potential of skeletal muscle may last well into the phase of contraction. From what is known of the chemistry of muscle, an electrical potential might a priori be expected during the development of tension, since the reaction involves a transformation of electrolytes.

On the other hand, there has been a tendency to regard these occasional findings of late potentials as artefacts, due either to a disturbance in the lead-off circuits with contraction (Fulton, 1926) or to a potential developed passively in the muscle during the distortion (De Meyer, 1921). Judin (1922) recorded from skeletal muscle polyphasic potentials whose successive maxima he assigned to peculiarities in the conduction of the excitation; his "tertiary waves," however, seem to be associated with contraction.

The present work was stimulated by the demonstration, by Gilson, on heart strips, that under approximately monophasic conditions, the potential is maintained during systole. Accordingly, on the expectation that the same conditions might obtain in skeletal muscle, we undertook to find whether by modifications of the usual methods we could demonstrate such a potential. Further, on the inference that the contractile process might represent a different chemical event from the excitation process, we have attempted to devise experimental procedures which might affect two such processes differentially.

The first clear evidence with which we are acquainted, that a potential accompanies tension development in skeletal muscle is in a paper by Lee (1887). He recorded, by the differential rheotome and capillary electrom-

eter, diphasic and monophasic action potentials from the frog sartorius. Lee found that in muscles killed under one electrode, the resultant monophasic potential curve showed a low plateau which extended the falling phase of potential through the phase of contraction. He interpreted the diphasic curves as consisting of monophasic potentials from the proximal electrode, lasting throughout contraction, opposed by a spike, due to the potential at the other electrode, which divided the whole into two portions. Arbeiter (1921) has shown that in the heart, various reagents affect the potential and the contraction roughly to the same degree. Further citations are quoted by Einthoven (1925). He concludes (p. 125) that neither in the heart nor in skeletal muscle is the "negative current fluctuation . . . . caused by the excitation wave, but is closely connected with the contraction itself."

In contrast to the idea that a nerve-like conduction process and a contraction process might each give rise to a potential, Fredericq (1921) has suggested that two different elements in a muscle might conduct impulses separately, at different rates, or with different rates of reaction.

1. *Potentials from diphasic leads.* We recorded the potential of skeletal muscle by means of the cathode ray oscillograph connected somewhat similarly as arranged for nerve (Erlanger, Bishop and Gasser, 1926). In view of the rapid fatigue of muscle under repeated stimuli, we have also made records of single twitch potentials. In this procedure, the rotating circuit breaker was replaced by a double contact key. The key is activated by means of a spring, whose motion is damped by an attached disk working into a bath of heavy oil. The breaking of the first contact opens a short circuit of the condenser whose charge moves the oscillograph beam along the time axis. The breaking of the second contact in the primary circuit of an induction coil causes the muscle to be stimulated. The muscle process is so slow that a single passage of the oscillograph beam across the recording photographic film gives a readable exposure. As leads, calomel electrodes were employed with yarn wicks.

Diphasic potentials from a green frog sartorius at 20°C. are shown in figure 1 (repeated stimuli) together with incompletely monophasic records, obtained by a technique to be described below (see fig. 8). The diphasic records often show an unmistakable second diphasic wave similar to a T-wave in heart muscle, and as shown in figure 2, such diphasic records can be reconstructed from the monophasic records. It is only necessary to assume that the process under the second electrode, farther from the stimulus, is of lower amplitude than that under the first, presumably due to the fact that not all the fibers run the full length of the muscle. The second process is therefore shunted by the inactive fibers.

In figure 3, *A*, the action potential of a dog sartorius is recorded diphasically, after cooling to 12°C. The conducting distance being 105 mm.

it was hoped that the two phases could be entirely separated. This did not occur, because with slower conduction in the cooled muscle, the potential also lasted longer. The figure records the first five responses after a period of rest. Owing to a slight shift of the base line as the amplifier adjusts itself to the changed input, these five records are more or less dis-

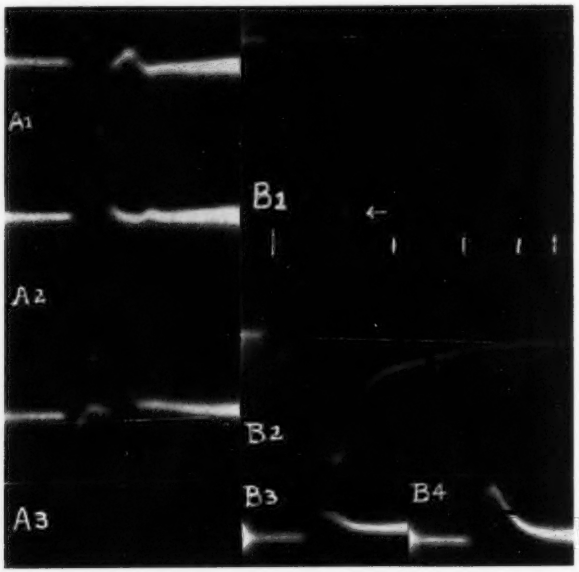

Fig. 1A. Records (repeated stimuli) of green frog sartorius with blood supply intact, recorded from the surface of the muscle. For method see figure 8, where leads 0 with 1, 2, 3, and stimulus positions 1′, 2′, 3′ apply to corresponding records 1, 2, 3. First phase down, second phase up, the preliminary spike being the shock effect. First two records diphasic, third partially monophasic. In front of the main wave of the third record are minor waves led off by contact of the muscle with the underlying tissues. In the first two records, the second diphasic waves presumably correspond to the T-wave of heart muscle, being too late for a conducted first process. The notch following the first crest represents the crest of the second wave, conducted to the more distal electrode. In record 3, the second phase predominates. 1B. Records, as above, bullfrog sartorius in body. First record, polyphasic potential superposed upon monophasic curve, due to effective local leads to underlying tissue during conduction. Leads 0, 3, figure 8, stimulus at 2′. Second record, monophasic lead obtained by killing so as to confine the excitation to the peripheral end of the muscle, which was freed from the body and supported by one electrode. Third and fourth records, shock alone, first weak, and second, just below threshold of second record. The shock potential is recorded downwards, the counter E.M.F. of polarization by the shock upwards, the action potentials downwards opposed to the underlying polarization effect. See figure 8, legend. Time in 0.01 second.

tinguishable. It can be seen first, that the second phase is lower in amplitude than the first, and second, that after the initial crest, the potential does not return at once to the base line. Further, this plateau following the excitation changes rapidly with fatigue, both in amplitude and in slope. In figure 3, *B*, the same muscle is recorded diphasically, as above, after partial fatigue, under repeated stimuli. This muscle previously recorded

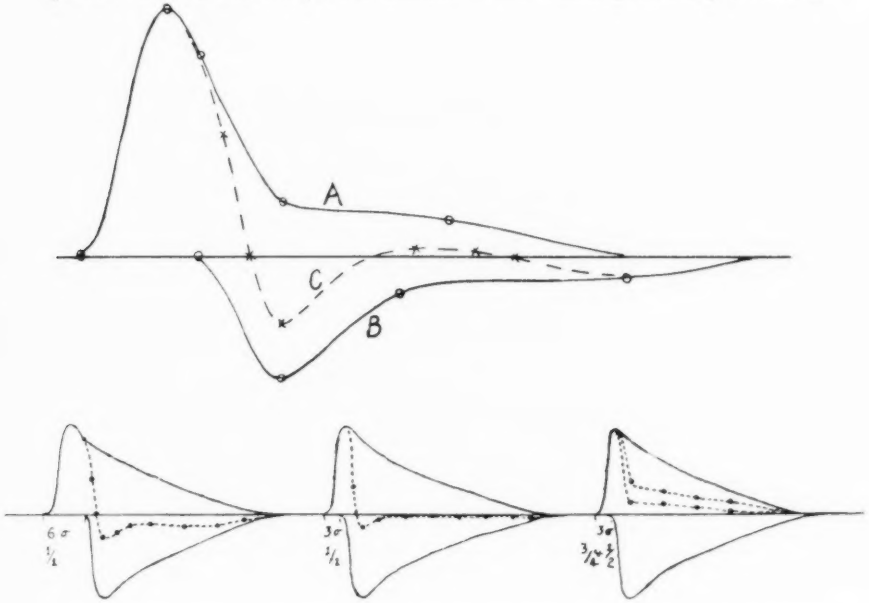

Fig. 2. Reconstructions, assuming a form for the monophasic potential as in figure 5 or 7, and 3 m.p.s. conduction rate, and that the second phase is depressed possibly by failure of some fibers to conduct throughout the muscle. The first phase is drawn to scale above the base line, the second at the stated fraction of the amplitude of the first below the base line, and later by the conduction time corresponding to the conducted distance chosen. The appearance of the "T-wave" or of the notch representing the apparent end of the "R-wave", depends on the form and amplitude of the two phases.

in the body with blood supply intact, showed essentially the same form of potential, but complicated by polyphasic waves due to multiple leads where the muscle made contact with underlying tissues. In the above records, and in those that follow, the contractions were as nearly isometric as possible, and the muscles were at least as extended as when in the body, generally more so.

2. *Conditions governing the form of the monophasic record.* The usual

method of killing under one electrode does not give the most accurate record obtainable of the potential change at the other electrode. The two possibilities of error seemed to be, first, the potential wav might be recorded at a distance from the electrode along the muscle surface, and, second, a potential might be recorded as if at the electrode on killed tissue, which really developed at the killed *ends* of the *live* fibers. It is obvious that the record is the algebraic sum of the potentials at the two electrodes. As will appear later, even with the muscle killed under one electrode, an action potential,

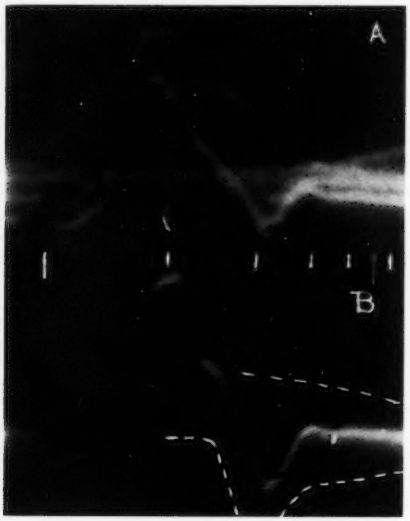

Fig. 3. Dog sartorius at 12°C. 105 mm. distance between leads, diphasic, except that all fibers may not have conducted the whole distance. A, five successive stimuli, the first two and last two waves superimposing. Taken after a period of rest. B, repeated stimuli to partially fatigued muscle. Dotted lines indicate probable course of two phases which sum to the record obtained. The plateau following the first peak of the first response falls rapidly in amplitude with successive stimuli, and is cut short by the second phase. Time in 0.01 second.

somewhat depressed in amplitude, may be recorded as if from this point, though in fact from the live margin of the tissue. This is not a mere spread of current from the action potential at a distance from the electrode. This is evident from the fact that, during conduction toward the electrode, the main potential wave is not recorded, after stimulation, until a time has elapsed adequate for such conduction (at about 3 m.p.s., see fig. 7*A*). The same phenomenon appears in nerve though not perhaps to so marked a degree. In a previous paper (Bishop, Erlanger and Gasser, 1926) it has

been stated that, since the demarcation potential caused a current to flow in a circuit from live tissue, around through dead tissue, and back into the live tissue, the killed tissue, and thus the lead taken from it, is effectively on some middle point of the potential gradient so caused. The action potential wave when it reaches the edge of the live tissue reduces the potential causing this gradient, and thus causes a change in the effective potential between live and killed regions, and thus between leads. The potential change so recorded is less than the action potential of the live tissue (though it may have the same form) both because the live tissue tends to be depressed in function near the killed end, and because the potential change recorded from the dead end is only a fraction depending on the effective position of the lead on the potential gradient between outside and inside of the fibers, through the dead region.

Now if the distance from the lead on live tissue, to the killed end which is the actual source of the potential change at the other lead, is great, the two phases of the potential as recorded will be separated by the conduction

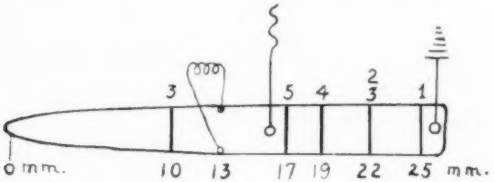

Fig. 4. Diagrams of lead *a* in text, and of leads for records of figure 5, q.v.

time of the impulse, while if this distance is small, the two phases will be nearly coincident. In the first case, the second phase tends to obliterate the last portion of the first phase. In the second case, its effect is to lower the first phase everywhere proportionally, and thus the form is not materially changed. Thus the greatest amplitude of the potential maximum will be obtained when the killed point is far from the live lead, but the most accurate form of potential when the muscle is killed close to the live lead. The distance from the live-dead margin to the lead on the killed tissue is not so important, and an extrinsic effect in the usual sense, as a spread of current from an excited region to an electrode at a distance from it, is not of appreciable magnitude.

Another circumstance renders the latter part of the action potential easy to be obscured. This is the form of the electrical wave itself in skeletal muscle. The potential following the first maximum falls gradually to the zero line. Where the second phase of a partially diphasic record follows the first in time, and is depressed in amplitude, the falling potentials, opposed to each other in the record, tend to be everywhere equal

in amplitude, and therefore balance out, as in figure 2. A T-wave may be almost absent, or a partially diphasic record may appear to be a simple monophasic wave without prolonged fall of potential.

3. *Records of monophasic potentials.* *a.* A straight fibered frog muscle (sartorius, gracilis, pectoral) was extended isometrically, stimulated at one end, and one lead electrode was placed close to the stimulating cathode (fig. 4). The platinum stimulating electrodes were placed on either side the muscle, the current passing in general transversely across it. Appar-

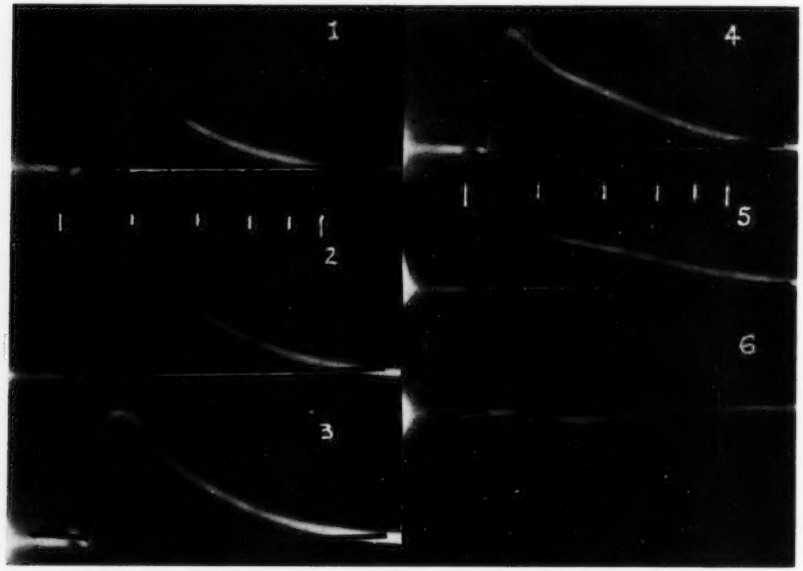

Fig. 5. Green frog sartorius, repeated stimuli, arranged on electrodes as in figure 4. Records 1 to 5 correspond to numerals 1 to 5 in figure 4, the perpendicular lines representing the corresponding region at which the muscle was killed. 6, shock alone. See text. Time in 0.01 second.

ently sufficient current spreads longitudinally in the fibers to give effective stimuli with shocks of moderate intensity. Owing to the symmetrical placing of anode and cathode with respect to the leading off electrodes, the shock effect on the records may be made small. The second lead electrode was tied to the other end, the muscle being killed by pinching just proximal to this. The live side lead was made in some cases by allowing a fine strand of yarn to touch the surface of the muscle at the site of stimulation, in other cases the yarn was tied firmly around the muscle. No difference was noted in the results. Records were now made after killing the muscle

at different distances from the stimulus, until not more than five millimeters of live tissue remained between stimulus and killed end (fig. 5). The result of this procedure is to shorten the time between the development of the potential at the first lead and at the killed region. Finally the muscle was killed between stimulating electrode and lead, both electrodes being left on (partially) live tissue, and a record taken of the shock escape (*5*, record 6).

As the distance between the live tissue lead and the killed region decreases, *the latter portion of the potential wave accompanying the contraction*, becomes greater in amplitude, until the live region is so short that it may be inferred that depression of function is taking place throughout the strip. Even then the latter portion of the potential is *relatively* still further increased by further killing. The strip is now so short that conduction of the impulse along it cannot account for the relatively long duration of potential, and the contraction is so slight that movement of the tissue with respect to the electrode cannot account for it either. The only other artefact that occurs to one is, an abnormally long reaction in injured tissue,

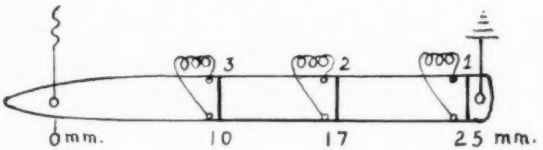

Fig. 6. Diagram of lead *b* in text, and for records of figure 7, q.v.

and this is excluded in other experiments. Stretching the muscle mechanically without stimulation (De Meyer, loc. cit.) gives a potential too low to be significant in these records.

b. Instead of recording the first phase and depressing the second by killing distally, the first phase may be depressed and the second recorded after conduction (fig. 6). This has the advantage that there can be no question of injury at the recording lead, and if the second lead is at the end of a muscle, the possible effect of an impulse conducted beyond it is obviated. When the first phase is the one to be observed, a second depressed wave, if present, would oppose the later low portion of the first. In the other case, the first depressed wave would oppose the early high portion of the second, and thus be less effective in obliterating the late low part of the record of the second phase. This technique involves stimulating the muscle between the recording leads, but if the stimulating electrodes are placed on either side the muscle, they are symmetrical with respect to the leads and the record of the shock may be made insignificant by adjustment of the position of the stimulators. The muscle strip becomes in effect a Wheatstone bridge, analogous to two parallel slide wires

joined at the ends to which current is led from the two sliders. A balance is detected from the two ends, and the balance is arrived at, as in any bridge circuit, by adjusting the ratio between two adjacent arms, i.e., sliding one stimulator along the muscle. Now the muscle is again killed shorter, and a series of records made with decreasing conducted distance from stimulator to killed end (fig. 7*B*). As before, the sloping plateau succeeding the first

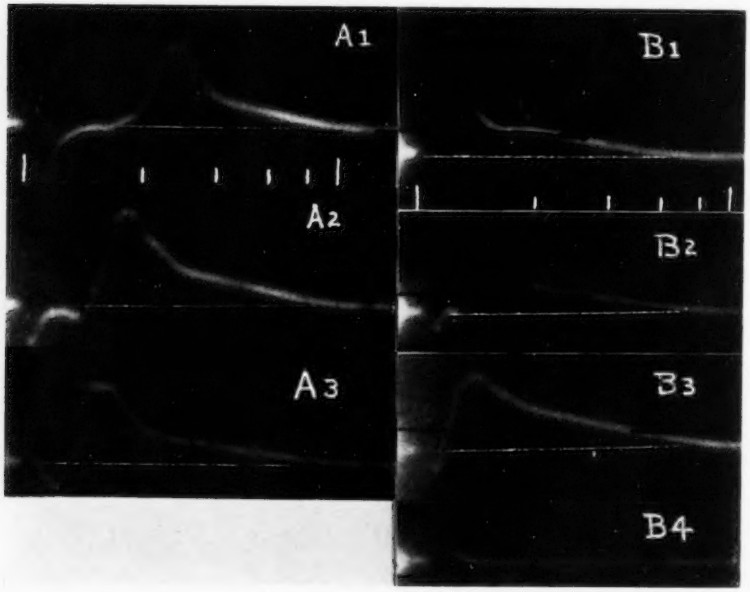

Fig. 7A. Bullfrog gracilis, repeated stimuli, arranged on electrodes as in figure 6, conducted distances as there noted. Stimulating electrode moved and muscle killed behind it. Shock record upward, too fast to record, polarization downward, complicated by a negative wave recorded as from the killed end, but originating at the live margin close to the stimulator. See text. Time in 0.01 second.

7B. Bullfrog gracilis, as in 7A, except that position of stimulus is at 3rd position, close to leading off electrode. Muscle killed successively shorter, as before. See text.

potential maximum increases relatively as the conducted distance to the killed region decreases. If instead of decreasing the conducted distance to the killed end, the distance to the lead is varied, and the distance from stimulator to killed end kept constant (fig. 7*A*) the change in the form of the potential is relatively slight, as should be expected.

c. A somewhat similar lead may be made from the muscle in the body with blood and nerve supply intact. The indifferent lead is placed any-

where on the body and the active lead is applied preferably away from the motor point to avoid confusing double stimuli. The action potential is recorded as the impulse passes the electrode, though before or after this passage, small waves of opposite sign may be recorded (fig. 1, *B*) at other effective points of contact of the muscle with the body, such as at the entrance of the artery. These, however, tend to be shunted out. The stimulus is applied across the muscle as previously. The action potential is monophasic in general, and approximately like that of a freshly removed muscle, except for secondary polyphasicity as noted above.

4. *Correlation between tension development and potential.* A natural inference to be drawn from the muscle potential curve is that the first maximum represents a process of excitation analogous to that of nerve, the subsequent plateau the process underlying mechanical contraction,

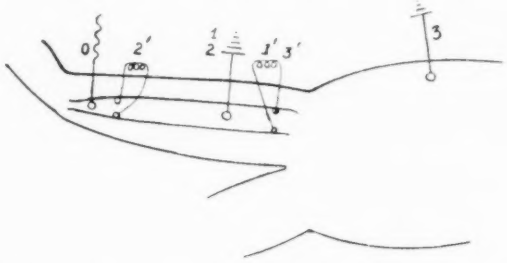

Fig. 8. Diagram of lead *c* in text, and for records of figure 1 as indicated. Numerals 1 and 1′, 2 and 2′, etc. refer to position of lead and stimulus, respectively, in the corresponding records of figure 1A. Lead at 0 is constant for all records. For 1B, leads for first record at 0, 3, stimulus at 2′. For second record, same arrangement, but muscle pinched behind 2, just before recording, the electrode lead at 0 raising the freed live end of the muscle off the body.

a differentiation contrary to the recent conclusions of Einthoven (loc. cit.). Now the process underlying contraction is the development of tension, depending on a glucose metabolism that might very well be electrochemical. The development of tension has recently been studied in the frog sartorius by Gasser and Hill (1924). They find, by following the effect of stretching during the contraction, that the maximum of the tension so elicited falls early in the contraction phase, and has decreased by the time that maximum shortening of the muscle takes place. They attribute the lag of contraction behind the alteration in elasticity to work done in distorting the plastic muscle substance.

If we subtract, from a monophasic potential wave, a potential curve with the form and the time relations of the curve of "internal response" of Gasser and Hill, the remainder is not far from the general form of the mono-

phasic potential of nerve, though its duration is longer. The form of curve here recorded is thus not inconsistent with the theory of dual functions of excitation and tension development, each with the characteristic potential.

The potential accompanying contraction might thus be that associated primarily with the reactions underlying development of tension, rather than with the maintenance of contraction or distortion of form, for the electrical sign decreases with time after the early maximum of internal elasticity change, and before the maximum shortening. The maximum of Gasser and Hill's curve falls upon the sloping plateau just after the first peak of the electrical response. If a process representing the nerve-like conduction in muscle, and the process underlying contraction, are in any sense distinct and separate, the electrical record may consist of the sum of two curves corresponding to these. Then the maximum of the second electrical process must fall at about the same time as the maximum of elasticity change, for the combined curve to show the form recorded. The latter potential lasts in general until after the crest of the contraction, and approaches zero asymptotically at some time during the phase of relaxation.

5. *Differentiation of two potential factors in muscle.* In general, it has been found previously that the action potential of muscle disappears pari passu with the ability to contract. Einthoven (loc. cit.) has particularly emphasized the idea that the two are inseparable. Certainly in fatigue this is the case. It might be expected that whatever prevented the irritable mechanism from functioning should prevent the subsequent contraction, but it does not follow that factors which depress the contractile function necessarily destroy irritability for conduction of any impulse. If the electrical sign of muscular response represents the sum of two components associated with two distinct functions, it should be possible to find reagents that would affect them differentially. The effects of saponin in Ringer's solution and of distilled water have been investigated with this consideration in mind.

A sartorius from a bullfrog is mounted in the usual manner, with a leading off electrode on either end, and a pair of platinum stimulators across the middle (fig. 6).

The application of saponin in dilute solution to the surface of a muscle causes contracture, but, according to Overton (Heffter, 1924) the saponin penetrates very slowly, only the surface fibers being affected for some time. However, a muscle so contracted, when stimulated, shows no further shortening, though it may be shown to conduct an impulse by leaving one end untreated, and stimulating the treated end. If now the muscle be mechanically extended, the treated section contracts violently in response to stimulation. Presumably the inside fibers are unaffected, but by contracture of the other fibers are passively shortened beyond their ability to shorten further.

Action potentials, led from muscles in their normal condition are similar to those led from muscles contracted by treatment with saponin until they show no further contraction upon stimulation. Both show a long lasting potential, decreasing somewhat in amplitude in the saponin treated muscle (fig. 9A). The latter shows a well-developed electrical potential during

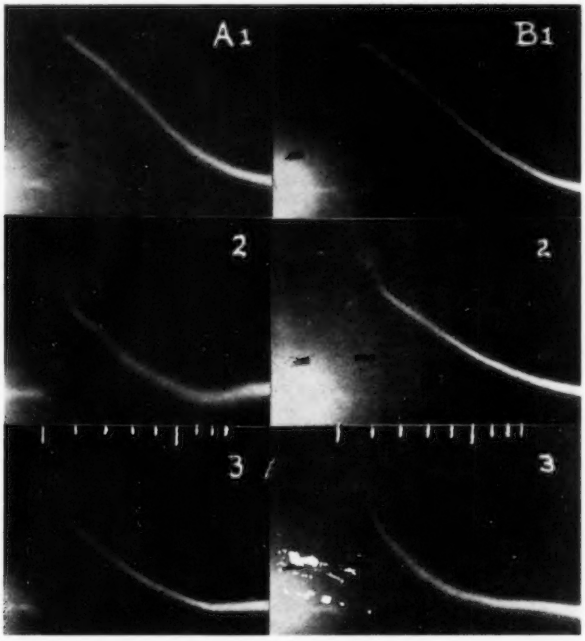

Fig. 9A. Bullfrog sartorius muscle, isometric, records of single twitches treated with dilute saponin in Ringer's solution: lead *b*, as described in text and figure 6. First record, untreated muscle, second record, maximally contracted by saponin, third record, same, muscle isometrically extended to allow of tension development.

Fig. 9B. Companion muscle to above, same leads, treated with distilled water. First record, untreated muscle, second, after 10 minutes' washing, third, after 30 minutes. The shock is up in all records, being too fast to record distinctly with one passage of the oscillograph spot. The first spike of the potential rises out of the falling phase of the shock. Conducted distance 15 mm. Time in 0.01 second.

what would be the contractile phase if contraction occurred. Now if the muscle is stretched, the action potential is not appreciably altered, but vigorous contraction occurs. This indicates that the electrical sign is not associated either with the performance of external work, nor with physical distortion or change of form, nor with mechanical changes in the recording

circuit due to shifting electrode contact. It may be a sign of something occurring in the muscle of an electrochemical nature, associated with the ability of the muscle to develop tension. As the saponin destroys more fibers, the whole potential becomes lower, but relatively little differentiation occurs between two elements of the electrical process. In general in a healthy muscle, the initial tension under which the muscle is put seems to have little effect on the form of the potential, until the tension is very high.

It has long been known that a water-logged muscle is able to conduct an impulse to a normal part, even when the treated part does not contract noticeably. It cannot be excluded, however, that the contractile mechanism is slightly functional, so that this experiment in itself does not dissociate conduction of an impulse from contraction. However, when potentials are recorded from such a muscle during water treatment, the relative change in the different portions of the potential wave is considerable. The muscle is killed at one side of the stimulators, and distilled water allowed to drip on the electrode and flow over the live region. The muscle promptly becomes white, swells and shortens. As this takes place, the later portion of the potential becomes progressively lower, with little change at first in the amplitude of the early peak (fig. 9*B*, 10). Even when stretched, such a muscle fails to contract. After 20 minutes' washing the peak of the potential may have decreased not more than 20 per cent, but instead of a following plateau, the potential drops rather rapidly to zero.

We have here at least a partial differentiation between two potential effects, which we believe indicates a dual character of the phenomena concerned. If so, it calls for a reconsideration of the hypothesis that ability to contract and action potential are inseparable. Tension development appears to be associated with the second potential effect, and *may* be separated from that function of muscle which corresponds to conduction in nerve. Other methods may be found for differentiating between these functions, and the above experiments are preliminary to a study of the effects of salt concentration, upon tension and potential, which work will be reported separately.

It has been stated above that the plateau following the initial peak of the potential falls off rapidly with fatigue. It appeared possible that in the water-logged muscle we were observing merely an accentuation of fatigue effects, due to stimulation to obtain a series of records. As far as the above conclusions are concerned, it makes little difference what is the cause of the differentiation. However, we have ascertained that the plateau can be depressed without obliterating the initial peak, by treating the muscles without stimulating them, and recording potentials during recovery. One of a pair of bullfrog sartorii was placed in distilled water, the other in 4 per cent sugar in distilled water, and left until the muscle in water showed no potential upon stimulation, while that in sugar showed

mainly an initial peak. The muscles were then put in Ringer's solution and their potentials recorded during recovery. The muscle from water recovered only partially, and very slowly, developing first an initial peak and later a rather low plateau; contraction returned with the latter. The muscle in sugar recovered more rapidly, and more completely, the peak of the potential returning to about the value that similar muscles have shown, but the plateau recovering less completely. Contraction recovered in this case also with its corresponding potential sign (fig. 11).

Discussion. While we feel satisfied as to the general validity of the findings reported above, we wish to make certain reservations as to the precision with which we can record muscle action potentials at present. Aside from the technical difficulties mentioned, there is the "physiological" difficulty that different muscles, treated apparently alike, differ markedly not only in the amplitude of the potential recorded, but in the relative amplitudes of the crest and the following fall. Compared to the variability of muscles, the potentials of nerve, for instance, appear rather startlingly mechanical. In muscle, it is apparently the falling phase of potential which is particularly susceptible to changes in the environment, the first maximum in this respect, as well as in others, more resembling nerve. In this connection, the bullfrog sartorius appears to be a much more rugged muscle than that of the greenfrog, in all respects.

In view of the fact that in nerve, different fiber groups are known to conduct at different rates, it might be questioned whether the same phenomenon in muscles would cause a complication of the picture. Also, since in our preparations to date the muscles have been neither curarized nor denervated, we presume we have often been stimulating fibers at one point directly, and at another indirectly. However, it is easy to show that neither of these factors can have an appreciable effect on the gross form of the potential wave. For at a conduction rate of 3 m.p.s., using bullfrog muscles up to 40 or 50 mm. in length, killed in the middle, the total conduction time cannot be over 6 or 8 σ, while the time to maximum of the potential as recorded is usually about 3σ. If the slower conducting fibers were half as fast as the faster, this would bring them 3 or 4σ later, and should make a difference in time to maximum of not more than half that. Because of the shortness of the conducted distance and the long duration of the potential (50 to 70σ) as compared to nerve, this factor is relatively unimportant. We presume that certain polyphasic records we have obtained are to be explained by these factors, but the successive crests of such records are all early in the total process. As the conducted distance is decreased, these successive maxima tend to disappear, leaving only the first, with no significant change in total duration of the complete potential wave.

We have also checked this possibility by recording potentials from

shocks below maximal. If some fibers conduct more slowly than others, they might be expected to have different thresholds corresponding, as in nerve (Erlanger and Gasser, 1924). Potential waves of maximal responses, cut down to one-half amplitude by suitable resistance shunts, usually exactly superimpose upon potentials of submaximal responses of the same unshunted amplitude. However, we have occasionally obtained records

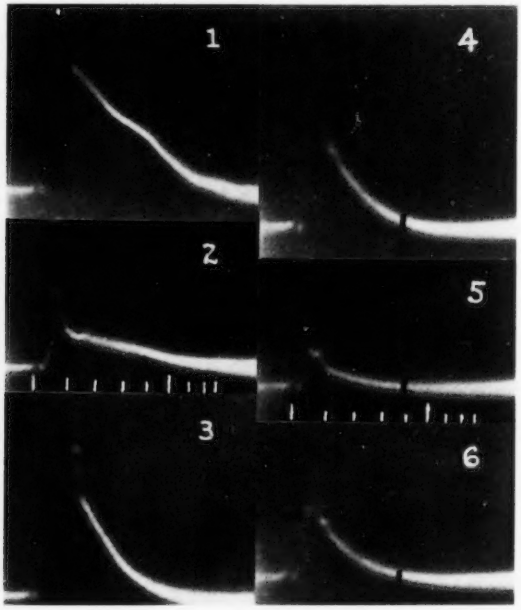

Fig. 10. Bullfrog sartorius recorded as in figure 9. 1, normal muscle; 2, submaximal stimulus; 3, water, 8 minutes; 4, water, 17 minutes; 5, submaximal stimulus, water 30 minutes; 6,maximal, water 30 minutes. The shock escape record is here insignificant, and shows as a break in the base line preceding response. The first peak of the potential of records 1 and 3 passes off the top of the records, involving too rapid a motion of the oscillograph spot to record with one passage. The actual form of 1 is thus that of 2, except for amplitude.

in which differences in stimulus strength affected the relative heights of peak and plateau, and we have no present explanation to offer for this. They are obtained under conditions where conduction in different fibers at different rates cannot be a factor, and suggest a variation in response with variations of the stimulus, near the stimulated point.

Our most satisfactory records have been obtained by the method *b* described above. When applying an electrical stimulus *between* the

recording leads, the conditions are exceptionally suitable for recording artefacts due to the shock itself, and we have checked the appearance of the shock, below threshold, in all experiments. The shock potential rises very abruptly, and falls decrementally, being virtually over, for the air-

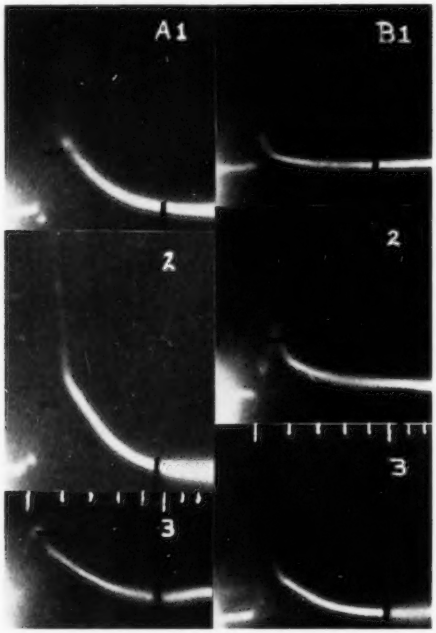

Fig. 11. Records of two bullfrog sartorii as in figures 9 and 10, but treated, A, with 4 per cent cane sugar in water for 30 minutes, and, B, with water. Records made during recovery in Ringer's without stimulation during previous treatment. The effect on the potential is due to the treatment, not to fatigue, and the muscles recover only partially. Record A, 1, in Ringer's solution, recorded immediately. 2. Ringer's 30 minutes, 10 mm. conduction. 3. Three hours later in Ringer's, action potential recorded at the stimulus, submaximal shock, with insignificant distortion in the rising phase too fast to record. Record B, 1 ,after water treatment, in Ringer's 5 minutes. 2. Ringer's 30 minutes. 3. Ringer's 45 minutes. 10 mm. conduction. Time in 0.01 second.

cored coils we have used, in less than 0.1σ. However, this potential record may be followed by a record of the counter E.M.F. of polarization due to the shock current (appearing as a wave of opposite sign) and this may last long enough to sum appreciably with the action potential. The shock potential is therefore approximately eliminated from the record by adjust-

ing the stimulating electrodes symmetrically with respect to the leads, as described, and when this is accomplished, the polarized areas being likewise symmetrically located, this counter E.M.F. record disappears also.

One other source of error lies in the change of potential due to movement of the electrode contact with contractions, noted by Fulton (loc. cit.). Such artefacts can be detected by the fact that they usually fall, not early in the muscle potential record, but late in contraction, and are generally diphasic and of long duration. They can be depressed by putting the muscle under reasonable tension, which does not materially affect the form of potential otherwise. The region where slightest motion should cause the greatest potential change is along the demarcation potential gradient near the killed end. If the muscle is killed in the middle, the "dead end" lead may be placed on live, unstimulated tissue beyond the killed region, and potential change due to change of electrode contact should here be a minimum. We suggest that a needle electrode thrust into the muscle is not the best type of lead, because the resultant injury should give demarcation gradients capable of producing such variation with movement. The De Meyer phenomenon may possibly be due in part at least to changes in resistance and effective lead contact along the killed-end demarcation gradient, upon passive elongation of the muscle.

It is hardly profitable to speculate at present on the significance of the action potential for the interpretation of muscle function. The form of the potential, however, and the amplitude, appear to be bound up with the conditions of muscle response, quite as in nerve, and may, with satisfactorily standardized technique for recording, serve to measure the changes in intensity and rate of the muscle processes under experimental conditions. The advantage of potential wave form as a record of muscle activity is freedom from complication by inertia, muscle viscosity and change in tension, beside the obvious technical precision of electrical methods. The second potential process may be a more precise measure of the essential tension-developing function of muscle than any other of its signs, and we infer that it is this potential, rather than the initial excitation potential, which may be coördinated with the initial heat development of Hill and his co-workers.

SUMMARY

1. Skeletal muscle action potentials when recorded diphasically may give second waves late in the contraction phase, apparently corresponding to the T-wave of the electrocardiogram. Polyphasic records may be obtained when effective multiple leads exist from the muscle to the electrode.

2. As in nerve, an undistorted record of one phase of the diphasic picture cannot be obtained by killing the tissue under the second electrode.

When the excitation reaches the killed end, a negative variation of the demarcation potential gradient, along which the effective killed end lead is made, is recorded at this lead.

3. When precautions are taken to minimize the above effect, and the potential is recorded near the stimulated region, it is maintained, after an initial maximum, at a considerable amplitude throughout the phase of shortening of the muscle. Fatigue by rapidly repeated stimuli depresses the later duration of potential more than it does the initial crest.

4. Certain considerations suggest that the latter portion of the potential may be an electrical sign of the chemical process underlying contraction, the initial maximum possibly representing the excitation process corresponding to that of nerve.

5. The form of the potential does not change with the initial tension nor with the mechanical distortion involved in shortening, when these are allowed to vary within reasonable limits. A partial differentiation between initial maximum and the subsequent prolonged fall may be brought about by washing to muscle in distilled water or sugar solution. The potential exhibited during the phase of contraction decreases with decrease of the ability of the muscle to develop tension. The initial potential crest is depressed later or less completely.

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